EVALUATION OF THE ROLE OF VITAMIN C IN CHRONIC BRONCHIAL ASTHMA

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment of the regulations

for the award of the degree of

M.D. (PHARMACOLOGY)

BRANCH – VI

GOVT. GE & HOSPITAL

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, INDIA.

MARCH 2010

CERTIFICATE

This is to certify that this dissertation entitled "**Evaluation of the role of Vitamin C in chronic bronchial asthma"** by the candidate Dr. K. G. Devibala for M.D. (Pharmacology) is a bonafide record of the research work done by her, under the guidance of **Dr. Usha Sadasivan M.D.**, **Ph.D.,** Professor, Department of Pharmacology, Stanley Medical College, during the period of study (2007 – 2010), in the Department of Pharmacology, Stanley Medical College, Chennai – 600001.

I also certify that this dissertation is the result of the independent work on the part of the candidate.

Dr. Usha Sadasivan M.D., Ph.D.
Professor
Department of Pharmacology
Stanley Medical College

Dr. S. MADHAVAN M.D.
Professor & Head of the Department
Department of Pharmacology
Stanley Medical College

Dr. S. CHITRA M.D.
Dean (i/c)
Stanley Medical College

ACKNOWLEDGEMENT

I express my sincere gratitude to Dr S. Chitra M.D., Dean (i/c), Stanley Medical College, and Dr. J. Mohanasundaram M.D., Ph.D., DNB, Former Dean, Stanley Medical College for permitting me to undertake this research work as a part of my MD curriculum.

I would like to convey my gratitude to Dr. S. Madhavan M.D., Professor and Head, Department of Pharmacology, Stanley Medical College for his sincere advice and constant support.

I owe my sincere thanks and appreciation to my guide, Dr. Usha Sadasivan M.D., Ph.D., Associate Professor, Department of Pharmacology, Stanley Medical College for her unfailing guidance, support and encouragement.

I thank Dr. S.Shivakumar M.D., Former Professor and Head, Department of Medicine, Stanley Medical College for permitting me to carry out the study in the Asthma Clinic OPD and Dr. Arvind M.D., Assistant Professor, Department of Medicine, for his unconditional co-operation and advice.

I express my sincere thanks to my Professors, Dr. B. Vasanthi D.O., M.D., and Dr. K. Vasanthira D.G.O., M.D. and Assistant Professors Dr. M. Kulandiammal D.G.O., M.D. and Dr. D. Jothi Lakshmi D.C.H., M.D., Department of Pharmacology, Stanley Medical College for their advice and encouragement.

I am extremely grateful to GlaxoSmithKline India Limited for providing me with drugs to conduct the study.

I thank Dr. A. Suguna Bai, Dr. B. Sharmila, Dr. J. Komathi, Dr. Deena Sangeetha C, former post graduates and Dr. R. Geetha, Dr. G. Sujatha, Dr. D. Arun Kumar, Dr. M. Mohanalakshmi and Dr. B. Kalaimathi, my fellow post graduates for their help and encouragement throughout this study.

I have great pleasure in thanking Mr. A. Venkatesan, Statistician, formerly in Stanley Medical College for helping me in the statistical analysis.

Finally I thank all my patients for willingly submitting themselves for this study.

ABBREVIATIONS

ACQ	-	Asthma Control Questionnaire
AIDS	-	Acquired Immune Deficiency Syndrome
bid	-	Two times a day
COPD	-	Chronic Obstructive Pulmonary Disease
CRP	-	C- reactive protein
DNA	-	Deoxyribonucleic acid
ELISA	-	Enzyme linked immunosorbent assay
EPO	-	Eosinophil Peroxidase
FEV_1	-	Forced Expiratory Volume in one second
FVC	-	Forced Vital Capacity
GINA	-	Global Initiative for Asthma
HIV	-	Human Immunodeficiency Virus
ICU	-	Intensive Care Unit
Ig	-	Immunoglobulin
IL	-	Interleukin
LABA	-	Long acting β_2 Agonist
MDA	-	Malondialdehyde
MPO	-	Myeloperoxidase
NADPH	-	Nicotinamide-adenine dinucleotide phosphate
Nk-κB	-	Nuclear Factor kappa-light-chain-enhancer of activated B cells
PAF	-	Platelet Activating Factor

PEFR	-	Peak Expiratory Flow Rate
PTA	-	Phosphotungstic acid
RAST	-	Radio allergosorbent test
ROS	-	Reactive Oxygen Species
SOD	-	Superoxide dismutase
TB	-	Tuberculosis
TBA	-	Thiobarbituric acid
TH_2	-	Type 2 T helper cell
tid	-	Three times a day

CONTENTS **PAGE NO.**

INTRODUCTION

Asthma is one of the major public health problems affecting 5% of the world population. It is a universal disease affecting people of all ages resulting in variable restriction to the physical, emotional and social aspects of an individual's life.

According to GINA (Global Initiative for Asthma), asthma is defined as
"a chronic inflammatory disorder of the airways characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli".

The major symptoms of asthma are paroxysms of dyspnoea, wheeze and cough, which may be mild and almost undetectable to severe and unremitting.

Asthma is a highly complex inflammatory disorder with many potential therapeutic approaches.Treatment with a combination of drugs which contain a corticosteroid and a long acting β_2 adrenoreceptor agonist is the most effective therapy.

Despite major advances in therapy, patient's symptoms are not adequately controlled.Recent evidence on the role of oxidative stress and inflammatory mediators has fostered considerable interest in new approaches for the treatment of bronchial asthma.

Epidemiological and observational studies suggest that increased oxidative stress or defective antioxidant status may be associated with an increased risk of asthma or faster disease progression.The generation of oxygen free radicals by activated airway inflammatory cells produce many of the pathophysiological changes associated with asthma.This suggests that antioxidants have a significant role in decreasing the incidence and severity of asthma.

Ascorbic acid or Vitamin C as it is commonly called is the major antioxidant present in the airway surface of the lungs suggesting a protective role of this vitamin against oxidative stress.

Studies have shown that Vitamin C intake in the general population correlates negatively with asthma.Patients with asthma may have low supplies of Vitamin C or increased demand for Vitamin C in the face of an oxidant load resulting in depletion of this vitamin.Hence, there is a need to clarify whether supplementation with antioxidants like Vitamin C may benefit in reducing the morbidity, improving the pulmonary function and quality of life in patients with bronchial asthma.

The purpose of this study is to determine the pathophysiological role of oxidative stress and the usefulness of antioxidant therapy using Vitamin C in patients with bronchial asthma.

REVIEW OF LITERATURE

DEFINITION

Asthma is defined as a disorder characterized by chronic airway inflammation and increased airway responsiveness resulting in symptoms of wheeze, cough, chest tightness and dyspnoea[1].

There are several components of airway inflammation in asthma like edema and denudation of the airway epithelium, activation of mast cells and infiltration with cells such as neutrophils, eosinophils and lymphocytes (TH_2 like cells) and collagen deposition beneath the basement membrane.

PREVALENCE

Asthma is a common disease,affecting approximately 5% of the population[2].It occurs at all ages but predominantly in early life. About one half of the cases develop before the age of ten and another half develop before the age of forty.

Men and women appear to be equally affected.In childhood, there is a 2:1 male preponderance, which disappears by adolescence and reverses after the age of 30[3].

In India, prevalence of asthma is reported to be 4%[4].

CLASSIFICATION

From an etiologic stand point, asthma is a heterogenous disease.For epidemiological and clinical purpose, asthma can be classified as follows.

I. ATOPIC OR EXTRINSIC ASTHMA

Atopy is the single largest risk factor for the development of asthma.It is often associated with

- Personal and /or family history of allergic diseases such as rhinitis, urticaria and eczema
- Positive wheal and flare skin reactions to intradermal injection of extracts of airborne antigens
- Increased levels of IgE in the serum
- Positive response to provocation tests involving the inhalation of specific antigen.

II. IDIOSYNCRATIC OR INTRINSIC ASTHMA

In patients with idiosyncratic asthma, there is

- No family history or personal history of allergy
- Negative skin tests
- Normal serum levels of IgE and hence cannot be classified on the basis of definite immunologic mechanisms.

They develop a typical symptom complex on contacting an upper respiratory illness.The initial insult may be little more than a common cold, but after several days the patient begins to develop paroxysms of wheeze and dyspnoea that can last for days to months.

III. MIXED TYPE

Many patients have disease that does not fit clearly into either of the above categories but instead falls into a mixed group with features of both.

In general, asthma that has its onset in early life tends to have a strong allergic component, whereas asthma that develops late tends to be non-allergic or to have mixed etiology.

PATHOPHYSIOLOGY

A.BRONCHIAL HYPER RESPONSIVENESS

Bronchial reactivity associated with airway inflammation is the common denominator underlying the asthmatic diathesis.The cause of this bronchial hyperreactivity may be genetic or acquired because of various allergenic and environmental factors.Several mechanisms may be involved such as airway epithelial injury, increased sensitivity to vagal reflex pathway, the release of arachidonate metabolites and the release of tachykinins from airway afferent nerves.

B.INFLAMMATORY CELLS

PRIMARY EFFECTOR CELLS

1. **Mast cells**

Recent evidence suggests that although mast cells are involved in the immediate response to allergens, they are unlikely to play an important role in the late response producing bronchial hyperresponsiveness, inflammation and chronic asthma.Inhaled allergens provoking an acute episode of asthma is unknown but seems to depend in part, on antigen antibody interactions on the surface of pulmonary mast cells with the subsequent generation and release of the mediators of immediate hypersensitivity.

2. **Eosinophils**

Eosinophil infiltration is a characteristic feature of asthmatic airways.Degranulation of eosinophils release major basic proteins which are toxic to the airway epithelium.They cause cilia to stop beating with exfoliation of cells in the bronchial lumen in the form of creola bodies.

3. **Lymphocytes**

Lymphocytes are prominent in asthmatic airways.Both T and B lymphocytes are involved in the production of IgE antibodies in response to various allergens.In addition, T lymphocytes also play a role in perpetuation of inflammatory response in asthma.

4. **Epithelial cells**

Epithelial cells may release inflammatory mediators like 15- Lipoxygenase which are chemotactic to other inflammatory cells.However, lack of epithelium which is due to the epithelial damage is an important factor in airway hyperresponsiveness.When epithelial cells are activated, they release a variety of mediators including leukotrienes, cytokines and PAF which could set up a self sustaining cycle enhancing bronchoconstriction.

5. **Platelets**

Platelets become activated by PAF released by mast cells and eosinophils.They may release a variety of mediators such as serotonin, thromboxane and lipoxygenase products resulting in bronchospasm and epithelial damage.

C.EPITHELIAL DAMAGE

Epithelial damage is a prominent feature of asthma and results in the presence of clumps of epithelial cells, i.e creola bodies.Epithelial damage is produced by basic proteins derived from eosinophils, release of oxygen radicals from inflammatory cells and as a consequence of submucosal edema.

This epithelial damage contributes to bronchial reactivity as the submucosal cells are exposed to antigens and other larger molecules resulting in further inflammation.

D.MICROVASCULAR LEAKAGE

Microvascular leakage is characteristic of inflammation in asthma.Leakage occurs at post capillary venules, followed by active contraction of endothelial cells by inflammatory mediators, thus allowing extravasation of macromolecules. Inflammatory mediators responsible for microvascular leakage include histamine, bradykinin, sulfidopeptide, leukotrienes and PAF.

E.MUCOSAL EDEMA

Microvascular leakage and inflammation lead to submucosal thickening and mucosal edema, which are responsible for increased airway resistance and bronchoconstriction.Mucosal edema, also contributes to epithelial shedding which is characteristic of asthma.

INFLAMMATORY MEDIATORS IN ASTHMA

Inflammatory mediators in asthma may have a variety of effects on the airways which may account for the pathological features of asthma.

Mediators such as histamine, prostaglandins and leukotrienes cause airway smooth muscles to contract, increase the microvascular leakage and mucus secretion and attract inflammatory cells.All these effects are mediated by interaction with specific receptors.It is possible that interaction among mediators accounts for bronchial hyperresponsiveness.Prostaglandin D_2 potentiates the bronchoconstricting response to histamine and cholinergic agonists in asthmatic patients, but this effect is transient.

DIAGNOSIS

CLINICAL DIAGNOSIS

The diagnosis of asthma is usually apparent from the symptoms of variable and intermittent airway obstruction but is usually confirmed by the objective measurement of lung function.

PHYSICAL EXAMINATION

Physical examination may be normal because asthma is an episodic disorder.The most commonly found abnormality on chest auscultation is wheeze, however normal chest auscultation does not rule out a significant limitation of airflow.

OBJECTIVE TESTING

I. LUNG FUNCTION TESTS

Simple spirometry confirms airflow limitation indicated by reduced FEV_1, FEV_1/FVC ratio and PEFR.Reversibility is demonstrated by > 12% or 200ml increase in FEV_1, 15 minutes after an inhaled short acting β_2 agonist or in some patients by a 2-4 week trial of glucocorticoids.

II. AIRWAY RESPONSIVENESS

The increased airway hyperresponsiveness is normally measured by methacholine / histamine challenge with calculation of the provocative concentration that reduces FEV_1 by 20% (PC_{20}).

III. HAEMATOLOGIC TESTS

(A) IgE

Total serum IgE and specific IgE to inhaled allergens (RAST) may be measured in some patients.

(B) C-reactive protein (CRP)

Measuring and charting C-reactive protein can prove useful in determining the disease progression or effectiveness of therapy in patients with bronchial asthma.

(C) EOSINOPHILS

Increased eosinophils may be observed in patients with asthma.

IV. IMAGING

1. ROENTGENOGRAPHY

Chest roetgenography is usually normal but may show hyperinflated lungs in severe asthma.In exacerbations, there may be evidence of pneumothorax.

2. COMPUTED TOMOGRAPHY

High resolution CT may show areas of bronchiectasis in patients with severe asthma and there may be thickening of the bronchial walls.

V. SKIN TESTS

Skin prick tests to common inhalant allergens are positive in allergic asthma and negative in intrinsic asthma.

ASSESSMENT OF ASTHMA CONTROL

The goals of asthma control (defined by the Global Initiative for Asthma – GINA)[5] are

1. Minimal (ideally no) chronic symptoms, including nocturnal symptoms.
2. Minimal (infrequent) exacerbations.
3. No emergency visits.
4. Minimal (ideally no) need for p.r.n. (as needed) β_2 agonist.
5. No limitations on activities, including exercise.
6. PEF circadian variation of less than 20 percent.
7. (Near) normal PEF.
8. Minimal (or no) adverse effects from medicine.

ASTHMA CONTROL SCORE

An Asthma Control Questionnaire (ACQ) was developed by Juniper et al (see Annexure IV). In patients whose asthma was stable between clinic visits, reliabilty of ACQ was high.The questionnaire includes a survey of important clinical symptoms and the use of short acting β_2-agonists as well as FEV_1[6].

TREATMENT

Some of the drugs that are effective in asthma can only be used via inhalation because they are not absorbed when given orally.Medications taken for asthma fall into two groups.

1. Relievers
2. Preventors

I. RELIEVERS

Relievers are rapid-acting bronchodilators that act to relieve bronchoconstriction and its accompanying acute symptoms such as wheeze, chest tightness and cough.

Inhaled β_2 agonists such as salbutamol are bronchodilators and act principally to dilate the airways by relaxing the airway smooth muscles.They reverse bronchoconstriction and related symptoms of acute asthma, but do not reverse airway inflammation or reduce airway hyperresponsiveness[7].

Long-acting β_2 agonists (LABAs), such as formoterol, salmetrol provide relief of symptoms in addition to a reduction in exacerbations[8].

II. PREVENTORS

Preventors are medications taken on a long term basis to keep persistent asthma under control.Of all medications, inhaled glucocorticoids are at present the most effective

controllers.Oral steroid medication is indicated in the treatment of an acute exacerbation of asthma or for long term treatment of unresponsive asthma[9].

Leucotriene receptor antagonists are another oral medication that can improve asthma control.More recently, Omalizumab (a recombinant humanised monoclonal antibody against IgE) has shown to be useful in patients with atopic asthma and concomitant allergic rhinitis[10].

OXIDATIVE STRESS

Many decades of research has produced a significant amount of data showing increased oxidative stress in asthma thus indicating a potential role for oxidants in the pathogenesis of the disease, particularly during exacerbations.

Putatively, relevant pro-oxidative mechanisms have also been identified.Currently available asthma drugs are generally effective for the treatment of the disease, but their effects on oxidative stress has still not been completely elucidated.

Oxidative stress is caused by a large variety of free oxygen radicals known as Reactive Oxygen Species (ROS), which includes

- Superoxide ($O_2^{\cdot -}$)
- Hydrogen peroxide (H_2O_2)
- Hydroxyl radical ($^{\cdot}OH$)
- Hypohalous acid (HOCl/HOBr)
- Peroxynitrite radical ($ONOO^-$)
- Nitric oxide (NO)

FREE RADICALS

DEFINITION

- "A free radical may be defined as an atom with one or more unpaired electrons, capable of independent existence".

- Atoms of transition metals such as iron and copper also contain an unpaired electron but are often not classified as free radicals.

- Free radicals can exist in liquid or gaseous phase, are potentially reactive with biological molecules and are usually denoted by the symbol (-)[11].

SOURCES

- Internal

- External

Internal sources

- ✓ Enzymatic reactions

- ✓ Exercise

- ✓ Inflammation

- ✓ Ischaemia/reperfusion

External sources

- ✓ Smoking
- ✓ Environmental pollutants
- ✓ Radiation
- ✓ Ultraviolet light
- ✓ Ozone

DISEASES ASSOCIATED WITH FREE RADICAL INJURY

a. Cancer

b. Atherosclerosis

c. Cerebrovascular accidents

d. Myocardial infarction

e. Senile cataracts

f. Osteoarthritis

g. Rheumatoid arthritis

h. Acute respiratory distress syndrome

i. Asthma and COPD.

MECHANISM OF FREE RADICAL INJURY

Excess free radical production leads to the following,

1. Lipid peroxidation of membranes
2. Oxidative modification of proteins
3. Lesions in DNA[12].

LIPID PEROXIDATION

- Lipid peroxidation is a process of oxidative decomposition of omega-3 and omega-6 PUFA of membrane phospholipids, leading to the formation of lipid hydroperoxides and aldehydic end products like malondialdehyde (MDA) and 4-hydroxynonenol.
- This process may cause disruption of cell structure and function and thus play an important role in the etiology of many diseases.
- Initiation and propagation of lipid peroxidation are mediated by free radicals.

MECHANISM LEADING TO LIPID PEROXIDATION IN ASTHMA

Oxidative stress, specifically lipid peroxidation is believed to contribute to the pathophysiology of asthma (Barnes Doelman et al, 1999)[13,14].

The innate and acquired immune system activates inflammatory cells and releases ROS that may overwhelm the host antioxidant defences and cause lipid peroxidation, accompanied by detrimental pathophysiological effects(Paredi et al, 2000)[15].

Exposure to a variety of substances such as allergens, gaseous pollutants, chemicals, drugs, bacteria and viruses lead to the recruitment and activation of inflammatory cells[16].

Allergen specific reactions involve the acquired immune system which is characterised by IL-5 production and subsequent recruitment and activation of eosinophils.In contrast, stimuli that act via the innate immune system leads to the production of IL-8 and subsequent recruitment and activation of neutrophils.

However, both these pathways lead to the production of ROS, primarily due to the respiratory burst of activated inflammatory cells which involves the uptake of oxygen and subsequent release of ROS into the surrounding cells[17,18].

(1) During the respiratory burst, a reduced nicotinamide-adenine dinucleotide phosphate-dependent superoxide-generating system is activated and releases superoxide ($O_2^{\cdot -}$) into the cell.

$$2O_2 + NADPH \xrightarrow{\text{NADPH oxidase}} 2O_2^{\cdot -} + NADP^+ + H^+$$

(2) A dismutation reaction catalysed by superoxide dismutase (SOD) then results in the production of hydrogen peroxide (H_2O_2).

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

(3) Hydrogen peroxide (H_2O_2) in the presence of halide ions (i.e. I^-, Cl^-, Br^-) will react to form hypohalous acid (e.g. HOCl/HOBr).This reaction is catalysed by eosinophil peroxidase (EPO) and myeloperoxidase(MPO) in eosinophils and neutrophils respectively.

$$H_2O_2 + Cl^- + H^+ \xrightarrow{EPO/MPO} HOCl + H_2O$$

(4) The hypohalous acid may then react with O_2^- or Fe^{2+} to produce another strong oxidant probably the hydroxy radical ($^{\cdot}OH$).

$$HOCl + O_2^{\cdot -} \longrightarrow {}^{\cdot}OH + Cl^- + O_2$$

$$HOCl + Fe^{2+} \longrightarrow {}^{\cdot}OH + Cl^- + Fe^{3+}$$

Thus, during this respiratory burst the inflammatory cells release high concentrations of $O_2^{\cdot -}$, $^{\cdot}OH$, HOCl/HOBr and H_2O_2 that may leak into the surrounding cells resulting in increased quantities of free radical in the airway tissues.

Furthermore, the inflammatory cells of asthmatics have an increased capability to generate free radicals which further contribute to high concentrations of Reactive Oxygen Species (Jarjour NN et al, 1994)[19].

- Another important reaction of biological concern is that between $O_2^{\cdot -}$ and NO resulting in the formation of peroxynitrite ($ONOO^-$), a potent oxidant capable of oxidizing reduced proteins, the polyunsaturated fatty acyl side chains of lipids and inducing the nitration of tyrosine.

- This reaction takes place in acidic conditions found in regions of inflammation and ischemia[20].

$$NO + O_2^{\cdot -} \longrightarrow ONOO^-$$

- The formation of this relatively long lived, strong oxidant from the reaction of NO and superoxide may contribute to inflammatory cell mediated tissue injury.

- Peroxynitrite (PN) is capable of oxidizing a variety of molecules.

  - High concentration of PN causes protein fragmentation.

  - PN initiates lipid peroxidation and this mechanism contributes to cytotoxicity mediated by oxygen and NO.

The cytotoxic effect of PN is protective when directed by inflammatory cells against invading micro-organisms and tumor cells[21].

ROLE OF NITRIC OXIDE (NO) IN ASTHMA

There is increasing evidence that endogenous NO plays a key role in the physiological regulation of airway functions and is implicated in airway diseases, including asthma.

- ✓ There is increased expression of iNOS (inducible Nitric Oxide synthases-major source of NO) in asthmatic airways, particularly epithelial cells and macrophages (Hamid et al 1993, Giaid et al 1998)[22,23].

- ✓ NO is a colorless, odourless gas that diffuses into airway lumen and can be detected in the exhaled air (Barnes, Kharitonov et al 1996)[24].

- ✓ The increased exhaled NO in asthma is related to airway inflammation (Jatakanon et al 1998)[25].

Thus, the excess quantities of Reactive Oxygen Species, that are produced by asthmatics may overcome the host antioxidant defences and cause oxidative stress.

EFFECTS OF OXIDATIVE STRESS

Oxidative stress can have many detrimental effects on the airway function, including

- Airway smooth muscle contraction (Rhoden KJ et al, 1989)[26]
- Induction of airway hyperresponsiveness (Weiss Katsumata U et al, 1990)[27,28]
- Mucus hypersecretion (Phipps RJ Adler KB et al, 1990)[29,30]
- Epithelial shedding (Doelman CJA et al, 1990)[31]
- Vascular exudation (Del Maestro RF Tate RM et al, 1982)[32,33].

Furthermore, ROS can induce cytokine and chemokine production through induction of the oxidative stress-sensitive transcription of nuclear factor-κB in bronchial epithelial cells (Biagioli MC et al, 1999)[34].

BIOMARKERS OF OXIDATIVE STRESS

» 8- Isoprostane

» Malondialdehyde

» Ethane

» Pentane

8-ISOPROSTANE

F_2 Isoprostanes are considered to be one of the important markers of oxidative stress as they are synthesized from arachidonic acid by free radical catalysed lipid peroxidation[35].Among the F_2 Isoprostanes, 8-Isoprostane is a reliable marker of oxidative stress and is increased in exhaled breath condensate, plasma and urine samples of patients with asthma[36].

MALONDIALDEHYDE (MDA)

The most commonly measured markers of lipid peroxidation are the aldehydes, MDA and 4 hydroxynonenal[37]. MDA is an end product of lipid peroxidation and is elevated both in plasma[38-41]and breath condensate of patients with asthma[42].

ETHANE AND PENTANE

Ethane and Pentane are produced by lipid peroxidation of n-3 and n-6 fatty acids and are elevated in breath condensate of asthmatics[43].

ANTIOXIDANTS

There are several mechanisms in the human body to counteract the damaging effects of free radicals.

- ✓ The first line of defence are the enzymes like glutathione peroxidase, superoxide dismutase and catalase, which require trace elements like selenium, copper, manganese and zinc for their activation.

- ✓ The second line of defence against free radical damage are the antioxidants like Vitamin C, Vitamin E, carotenoids, flavonoids, etc.

Some antioxidants like ubiquinone, glutathione and uric acid are produced during normal metabolism in the body. Others like Vitamin C, Vitamin E and the carotenoids can be obtained from the diet.

"An antioxidant is a molecule stable enough to donate an electron to a free radical and neutralize it, thus reducing its capacity for tissue damage."

Anti-oxidants can be classified into:

Primary Antioxidants

- Vitamin E
- Vitamin C
- Carotenoids
- Flavonoids
- Polyamine
- Melatonin
- Estrogen
- Ubiquinone
- Lipoic acid
- Uric acid
- Glutathione
- Superoxide dismutase
- Glutathione peroxidase
- Catalase

Secondary Antioxidants

- Copper
- Glutathione reductase
- Ascorbate reductase
- G6PD
- Cerruloplasmin
- Transferrin
- Metallothionein
- Albumin
- Bilirubin
- N-acetyl cysteine

ANTIOXIDANTS IN ASTHMA

Asthma is a condition involving chronic airway inflammation and oxidative stress.The lungs are frequently exposed to toxic oxidants from air pollutants, cigarette smoke or from reactive oxidants released by inflammatory cells during inflammation.

- ✓ Many controlled studies suggests that there is an antioxidant deficiency in asthma which indicates impairment in the pathways protecting lung cells from oxygen mediated damage[44-48].Barnes PJ et al, 2002 has shown a marked reduction in plasma antioxidant capacity during exacerbations of bronchial asthma[49].

- ✓ In stable asthmatics, decreased activity of copper and zinc containing superoxide dismutase in bronchial epithelial cells and bronchoalveolar lavage fluid cells has been found (Smith LJ et al, 1997)[50,51].

- ✓ A polymorphism in antioxidant enzymes, for example Mn-SOD and glutathione s-transferase has also been reported in asthmatic subjects (Hulsmann AR et al, 1994)[52].

- ✓ Similarly, peroxynitrite inhibitory activity, an antioxidant system is reduced in the sputum of patients with stable asthma and its level is positively related to airway responsiveness and negatively related to forced expiratory volume in one second (FEV_1) and the degree of sputum eosinophilia (Kanazawah H et al, 2002)[53].

- ✓ Among antioxidant systems, the cyclin dependent kinase inhibitor $p21^{CIP/WAF1}$, a protein that protects against oxidative stress, and extracellular glutathione peroxidase are increased in bronchial epithelial cells of asthmatic patients (Puddicombe SM et al, 2003)[54,55].

- ✓ A link between asthma and selenium deficiency (an essential element for the normal activity of glutathione peroxidase) has also been hypothesised (Omland O et al, 2002)[56].

Respiratory viruses represent the most important cause of asthma exacerbations.

- ➢ Rhinovirus, the most frequently identified virus in respiratory tract specimens in asthma causes intracellular oxidant generation.

- ➢ This is a crucial step in the activation of NF-κB and in the production of proinflammatory adhesion molecules and cytokines (Papi A et al, 2002)[57].

- ➢ Antioxidants inhibit both rhinovirus induced oxidant generation and inflammatory mediator production and release (Biagioli MC et al, 1999)[58].

Epidemiological evidence suggests that exogenous antioxidants have a significant effect on the incidence and severity of asthma (Smith Fogarty H et al, 2000)[59].Several studies have also demonstrated that antioxidants are able to decrease the airway inflammation and hyperreactivity in animal models of asthma (Cho Lee et al, 2004)[60].

Recent studies have suggested that consuming antioxidants such as Vitamin C, Vitamin E, β carotene, flavonoids, selenium and other nutrients reduces the risk of bronchoconstriction associated with asthma (Ford ES et al, 2004)[61].

Vitamin C is the major antioxidant present in the airway surface of the lungs where it may help to protect against the effects of exogenous oxidants such as cigarette smoke and endogenous oxidants such as those produced by inflammatory cells in individuals with ongoing asthma (Gary E Hatch et al, 1995)[62].

Hence, from the data available in the literature one can conclude that antioxidants particularly Vitamin C may have a potential role in the treatment of asthma, especially that of acute exacerbations.

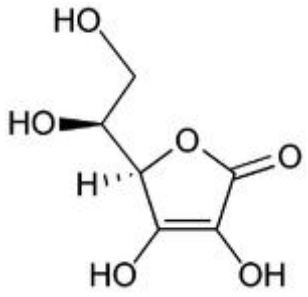
VITAMIN C

Vitamin C (L-ascorbic acid, ascorbate), a water soluble micronutrient is essential for human health.Vitamin C is synthesized by plants and most animals. Humans and other primates cannot synthesize Vitamin C because of lack of L-gulonolactone oxidase, the terminal enzyme in the biosynthetic pathway of Vitamin C from glucose.As a consequence, humans must obtain Vitamin C exogenously, usually from food.

HISTORY

In 1912, a Polish-American biochemist, Casimir Funk, while studying deficiency diseases, developed the concept of vitamins to refer to the nutrients which are essential to health.Then from 1928 to 1933, the Hungarian research team of Joseph L Svirbely and Albert Szent-Gyorgyi and independently the American Charles Glen King, first isolated Vitamin C and showed it to be ascorbic acid.For this, Szent-Gyorgyi was awarded the 1937 Nobel prize in medicine.

CHEMICAL STRUCTURE



Vitamin C (L-ascorbic acid, ascorbate) is a six carbon α ketolactone weak acid with a p_k of 4.2 and a molecular weight of 176.

SOURCES

- ✓ Fruits
    - Gooseberries
    - Guava
    - Lemon
    - Orange
    - Papaya

- ✓ Root vegetables
    - Carrot
    - Potato

- ✓ Fish and sea foods
- ✓ Milk and dairy products
- ✓ Cereals
- ✓ Pulses

PHARMACOKINETICS

Ascorbic acid is readily absorbed by active transport from the intestines.Following absorption, ascorbic acid circulates freely in plasma, leukocytes and red blood cells and is extensively distributed to all cells of the body.It has a plasma half life of 16 days.The main route of excretion of ascorbic acid is urine,oxalate being the main metabolite.

PHYSIOLOGICAL FUNCTIONS

Vitamin C is a highly effective antioxidant that is responsible for maintaining iron in its reduced state, thus preserving the activity of the enzymes that contain iron at the catalytic site.The well documented of these enzymes are the iron containing prolyl and lysyl hydroxylases that catalyze the post-translational hydroxylation of proline and lysine.Hydroxyproline and Hydroxylysine provide the site for cross-linking of collagen fibrils responsible for the tensile strength and elasticity of connective tissue.Tissues most sensitive to Vitamin C status are those which contain large amounts of collagen such as blood vessels and capillaries,bones, and scar tissue.

Vitamin C dependent reactions also include phagocytic activity, neurotransmitter synthesis, and hepatic production of bile from cholesterol.

It was also found that Vitamin C extends the activity of Vitamin E by reducing oxidized tocopherol so that Vitamin E can again function as an antioxidant.It also improves the bioavailabilty of inorganic dietary iron by maintaining the reduced form which is more soluble and readily absorbed.

INDICATIONS

Treatment of overt scurvy, or of Vitamin C deficiency status.

CONTRAINDICATIONS

Patients with

- Hyperoxaluria
- Glucose-6-phosphate dehydrogenase deficiency
- Iron overload

DEFICIENCY

SCURVY

Scurvy is an avitaminosis resulting from lack of Vitamin C.The earliest symptoms of scurvy are weakness and lassitude.Physical signs include

- Petechial haemorrhage
- Perifollicular hyperkeratosis
- Erythema and purpura
- Bleeding into the skin, subcutaneous tissues, muscles and joints
- Arthralgia and joint effusions
- Swollen and friable gums

ADVERSE EFFECTS

Vitamin C is generally safe and well tolerated with very few dose related side effects.

AT THERAPEUTIC DOSES

- Nausea
- Vomiting
- Diarrhoea
- Headache
- Flushing of the face
- Disturbed sleep

AT TOXIC DOSES (chronic administration of >10 g /day)

- Kidney oxalate stones
- Water and electrolyte imbalance
- Increased red cell lysis
- Rebound scurvy
- Suppression of cobalamin activity

THERAPEUTIC USES

- Prevention and treatment of scurvy
    - Prophylactic dose – 50-100mg/day
    - Therapeutic dose – 0.5-1.5g/day
- Postoperatively (500mg/day) to accelerate wound healing
- Anaemia: Ascorbic acid enhances iron absorption and is frequently combined with ferrous salts
- To acidify urine(1g tid) in urinary tract infections
- Protection against cancer and heart diseases
- Helps prevent cataract
- Assists in lowering blood cholesterol
- Prevents many types of viral and bacterial infections
- Acts as a natural laxative.

VITAMIN C AS AN ANTIOXIDANT

Vitamin C is essential for life and is a powerful water soluble antioxidant.It is an electron donor and therefore a reducing agent.All known physiological and biochemical actions of Vitamin C are due to its action as an electron donor.

Vitamin C is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized.However, by the very nature of this reaction, Vitamin C itself is oxidized in the process.

It is noteworthy that when Vitamin C donates electrons, they are lost sequentially.The species formed after the loss of one electron is semidehydroascorbic acid or ascorbyl radical, a free radical.

As compared to other free radicals, ascorbyl radical is relatively stable with a half life of 10^{-5} seconds and is fairly unreactive.This property explains why ascorbate may be a preferred antioxidant.In simple terms, a reactive and possibly harmful free radical can interact with ascorbate.The reactive free radical is reduced, and the ascorbyl radical formed in its place is less reactive.

Reduction of a reactive free radical with formation of a less reactive compound is called free radical scavenging or quenching.Ascorbate is therefore a good free radical scavenger due to its chemical properties[63].

A number of studies have investigated the effect of Vitamin C on chronic diseases associated with oxidative stress.

- The putative role of ascorbate in the management of AIDS is still unresolved for more than 16 years after the landmark study published in the proceedings of National Academy of Sciences (USA) showing that non toxic doses of ascorbate suppresses HIV replication invitro (Harakeh S et al, 1990)[64].

- In an animal model of lead intoxication, Vitamin C demonstrated the "protective effects" on lead-induced nerve and muscle abnormalities (Simon JA et al, 1999)[65].In smokers, blood lead levels declined by an average of 81% when supplemented with 1000mg of Vitamin C, suggesting that Vitamin C supplements may be an "economical and convenient" approach to reduce lead levels in the blood (Dawson E et al, 1999)[66].

- Small clinical trials have found that Vitamin C might improve the sperm count, sperm motility, and sperm morphology in infertile men (Akmal M et al, 2006)[67].

- In some observational studies, Vitamin C consumption from both food and supplements correlated with reduced mortality (Enstrom JE et al, 1992)[68] and with a lower risk of ischaemic heart disease (Osganian SK et al, 2003)[69].

- In a large placebo-controlled study, combined supplements of Vitamin C and Vitamin E reduced the odds of developing advanced age related macular degeneration (Janet W et al, 2001)[70].

- A preliminary study published in the Annals of Surgery found that the early administration of antioxidant supplementation using ascorbic acid and α-tocopherol reduces the incidence of organ failure and shortens ICU length of stay in critically ill surgical patients (Nathens A et al, 2002)[71].

- Vitamin C as a supplement was also tested for potential beneficial effects on respiratory diseases like asthma and COPD and has shown promise.

A randomized double blind, placebo-self-controlled cross over trial carried out at Tanta University in Egypt, headed by Mohammad Al Biltagi et al, 2009 has found that Vitamin C supplementation was associated with a significant improvement in asthma measures, lung function and markers of inflammation[72].

Research exploring the role of antioxidants in the prevention and management of asthma has dramatically increased in recent years.Because asthma is an inflammatory disease that has been associated with oxidative stress, it is plausible to consider the role of antioxidant supplementation as an alternative treatment for asthma.

Vitamin C is one of the key antioxidants which is abundant in the extracellular fluid lining the lungs and low Vitamin C intake has been associated with pulmonary dysfunction.

- ✓ The pooled results of 40 studies conducted between 1980 and 2007 showed that people with asthma have a significantly lower intake of Vitamin C (about half of the recommended daily intake).

- ✓ In addition, low circulatory levels of Vitamin C in the blood and lower dietary intake of food containing Vitamin C were associated with a 12% increased risk of asthma (Jo Leonardi Bee et al, 2009)[73].

- ✓ These results are consistent with the data obtained from the studies of

    - Ford ES, Rubin RN et al, 2004 who showed that Vitamin C supplementation can alleviate the severity of asthma symptoms[61].

    - Jaber R et al, 2002 found that 1 or 2 g of Vitamin C daily diminishes episodes of exercise induced asthma[74].

With increasing evidence on the role of oxidative stress in the pathogenesis and severity of asthma, it is imperative to study whether supplementation with antioxidant Vitamin C could offer benefit in the prevention and management of asthma.

OBJECTIVE

To study the beneficial effects of Vitamin C as an add on therapy to the standard drug therapy in patients with chronic bronchial asthma using

1. Asthma Control Questionnaire (ACQ) score for asthma control

2. Lung Function Tests

   - PEFR
   - FEV_1

3. Serum Malondialdehyde, a marker of oxidative stress

4. Serum C - reactive protein, a marker of inflammation.

METHODOLOGY

STUDY DESIGN

Phase III prospective, open, two arm, parallel group, out patient randomized, active controlled study.

STUDY CENTRE

Asthma clinic,

Department of Internal Medicine,

Stanley Medical College Hospital,

Chennai.

STUDY PERIOD

January 2008 to December 2008

STUDY DURATION

Active drug therapy-2 months

Follow up-1 month

} 3 months for each patient.

STUDY POPULATION

Patients attending the Outpatient Asthma clinic, Department of Internal Medicine, Stanley Medical College Hospital, Chennai.

SAMPLE SIZE

80 patients

40 patients in each group

STUDY PROCEDURE

The study was started after obtaining the approval and clearance from the Institutional ethics committee (Annexure-I).

INCLUSION CRITERIA

» Age group 18 – 60 years

» Both sexes

» Patients with bronchial asthma

» Duration of disease >5 years

EXCLUSION CRITERIA

» Age group < 18 years and > 60 years

» Patients with

- COPD
- TB
- Cardiac disease

» Smokers

» Pregnant and lactating women

ENROLLMENT VISIT

Patients who attended the Outpatient Department of Asthma clinic, Stanley Medical College Hospital were explained in detail about the study procedure, purpose and its benefits.

The purpose of this study was to

- ✓ Achieve better asthma control
- ✓ Reduce complications
- ✓ Improve the quality of life

Written informed consent was obtained from the patients willing to participate in the study, in the prescribed format in the regional language prior to the commencement of the study procedure.

If the patient was illiterate, the left thumb impression was sought.This was done in the presence of an impartial witness.

Patients were advised to come the next day at 8.00 AM for the screening procedure.

SCREENING

Patients who had given the written informed consent for participation in the study were screened by detailed medical history, objective measurement of lung function and physical and systemic examination.Baseline demographic characteristics were recorded.Blood was drawn for determining the haematological and biochemical parameters.

RECRUITMENT

80 patients who fulfilled the inclusion criteria were recruited for the study.5 more patients, than the required sample size were recruited to compensate for the dropouts.

RANDOMISATION

Among the 85 patients, all the odd number patients were given Vitamin C in addition to the regular medications (study group) and even number patients were given only the regular medications (control group).

DRUGS

- Tablet Vitamin C 500mg Indian Pharmacopoeia (Celin) was supplied by GlaxoSmithKline Pharmaceuticals Limited, Mumbai-400030.

- Standard drugs like tablet Salbutamol and tablet Aminophylline were supplied by the Pharmacy, Stanley Medical College Hospital, Chennai-600001.

DOSAGE AND ADMINISTRATION

CONTROL GROUP

Tab.Salbutamol 4mg b.i.d. + Tab.Aminophylline 100mg t.i.d. for a period of 8 weeks.

STUDY GROUP

Tab.Vitamin C 500mg b.i.d. + Tab.Salbutamol 4mg b.i.d. + Tab.Aminophylline 100mg t.i.d. for a period of 8 weeks.

STUDY VISITS

Patients were assessed once in two weeks for a period of 8 weeks.

VISIT I (BASELINE):

- Written informed consent
- Randomisation
- General medical history and physical examination
- Asthma Control Questionnaire (ACQ)
- Lung function tests-PEFR,FEV_1
- Serum Malondialdehyde
- Serum C – reactive protein
- Other routine haematological and biochemical parameters.

VISIT II (AT THE END OF THE 2^{ND} WEEK):

- Clinical examination
- ACQ
- PEFR

VISIT III (AT THE END OF THE 4^{TH} WEEK):

- Clinical examination
- ACQ
- PEFR
- FEV_1

VISIT IV (AT THE END OF THE 6TH WEEK):

- Clinical examination
- ACQ
- PEFR

VISIT V (AT THE END OF THE 8TH WEEK):

- Clinical examination
- ACQ
- PEFR
- FEV_1
- Serum Malondialdehyde
- Serum C – reactive protein

VISIT VI (AT THE END OF THE 12TH WEEK AND FOLLOW UP PERIOD)

- Clinical examination
- ACQ
- PEFR
- FEV_1

EVALUATION

I. ASTHMA CONTROL SCORE

Asthma control was assessed using the Juniper asthma control questionnaire (ACQ) on each visit.For the standard clinical version of the ACQ score, there are seven questions , each scored on a seven point scale (0 = good control, 6 = poor control).The overall score is the mean of the seven responses which include the following.

1. Nocturnal symptoms

2. Severity of asthma attacks

3. Limitation of activities

4. Shortness of breath

5. Frequency of asthma attack

6. Short-acting bronchodilator use

7. Forced expiratory volume in one second (FEV_1) / Peak expiratory flow rate (PEFR)

II. LAB INVESTIGATIONS

A. Haemoglobin

B. Total count and differential count

C. Bleeding time and clotting time

D. Random blood sugar

E. Blood urea

F. Serum creatinine

G. Urine analysis

H. Serum C - reactive protein

I. Serum Malondialdehyde

A. ESTIMATION OF SERUM C-REACTIVE PROTEIN

The subjects were asked to come to the Outpatient Department of Asthma clinic, Stanley Medical College Hospital at 8.00 AM on the sample collection day.On arrival, the subject's vital signs and compliance with regular medication were recorded and ascertained that the condition of the subject was normal.

The blood samples for determining serum C-reactive protein were collected by venepuncture.At each sampling 10 ml of blood was drawn.

All the vital signs were monitored at the end of the sampling and only when both the patient and the clinician were confident, the subjects were allowed to leave the Outpatient Department of Asthma clinic where the study was conducted.

The serum C-reactive protein level was estimated using enzyme linked immunosorbent assay (ELISA).The values are expressed in mg/L.

B. ESTIMATION OF SERUM MALONDIALDEHYDE BY DRAPER AND HADLEY METHOD

Reagents required

Phosphotungstic acid (PTA) 10%

Thiobarbituric acid (TBA) 0.67%

n-Butanol

Sulphuric acid 4M

MDA Standard (tetra methoxy propane in distilled water)

Procedure

0.1 ml of serum was mixed with 0.5 ml of sulphuric acid, 0.4 ml PTA and 1 ml of distilled water.The tube was centrifuged for 10 minutes at room temperature.The supernatant was aspirated and the remaining pellet was mixed with 1 ml sulphuric acid and 0.15 ml PTA.This was centrifuged for 10 minutes, supernatant was discarded and the pellet was resuspended in 2 ml of water.0.5 ml TBA was added and the contents heated in a boiling water bath for 60 minutes.

The tubes were cooled and 2.5 ml butanol was added.The tubes were centrifuged, the supernatant was added to the cuvette and the absorbance was measured at 533 nm.A standard calibration curve was prepared by taking various concentrations of MDA standard, treated similarly with TBA.

The values are expressed in nM/mL.

Normal value of serum MDA is 12-15 nM/mL

C. MEASUREMENT OF PEFR

The severity of bronchial asthma depends on the degree of bronchial obstruction and bronchospasm and is measured as Peak expiratory flow rate (PEFR).Thus PEFR by determining the degree of obstruction of airways aids in the diagnosis, assessing the severity and also monitoring the response to therapy.

Peak expiratory flow meter (pulmopeak) measures the peak expiratory flow rate (PEFR) which is a valuable indicator of lung function and meets the new technical standards established by the National Asthma Education Programme.

PROCEDURE

- Patients were asked to sit or stand upright and hold the peakflow meter with the thumb underneath the instrument and the other four fingers on top.
- They were instructed to inhale as deeply as possible filling their lungs with air and place their mouth on the mouthpiece, past their teeth and form a tight seal.
- Patients were then asked to blow out as hard and fast as they can,which moves the indicator level and shows the PEFR
- This procedure was repeated two or more times, the indicator automatically recording the best result.

D. SPIROMETRY

Spirometry testing was conducted with MIR SPIROLAB II spirometer with an attached microprocessor.

All testing was performed at the Department of Physiology, Stanley Medical College, using the spirometry standards of the American Thoracic Society.

PROCEDURE

- Before performing the test, spirometer calibration was checked.
- The procedure was explained in detail to the patients.
- Height and weight of the patients were noted
- Patients were instructed to assume the correct posture with the head slightly elevated
- They were instructed to inhale rapidly and completely, closing their lips around the mouthpiece forming a tight seal.
- Patients were then asked to exhale maximally until no air can be expelled while maintaining an upright posture.
- This manoeuvre was done thrice and tested for repeatability.

INSTRUCTIONS TO THE PATIENTS

- The patients were instructed to come fortnightly to collect the medication
- They were advised to take their medicines regularly, after meals
- The necessity for compliance to the regimen was explained
- Any intercurrent minor illness and medication taken for the same was to be reported at the next visit
- Any adverse events experienced by the patients were to be reported at the next visit
- The patients were advised to report to the hospital if they experienced any worsening of symptoms.

COMPLIANCE

Compliance was recorded by

1. Daily drug reminder chart (Annexure V)
2. Examining the number of unutilized capsules in each medication pack

FOLLOW UP

At the end of two months of active drug therapy, both the control and study groups were followed up for a further period of one month.

The results were subjected to statistical analysis.

RESULTS

- The data obtained at the end of this study was analysed using SPSS software.

- The following tests were used,

  - ✓ Student independent ‘t’ test
  - ✓ Student paired ‘t’ test
  - ✓ Chi square test
  - ✓ Oneway ANOVA F-test
  - ✓ Repeated measures of ANOVA test.

- P value ≤ 0.05 was considered significant.

AGE DISTRIBUTION

Table 1:

Group	n	Mean	Std. Deviation	Student independent t-test
Control	40	39.33	10.413	t=0.11 P=0.90 Not significant
Study	40	39.60	10.185	

There was no statistically significant difference between the study and the control groups.

Figure 1:

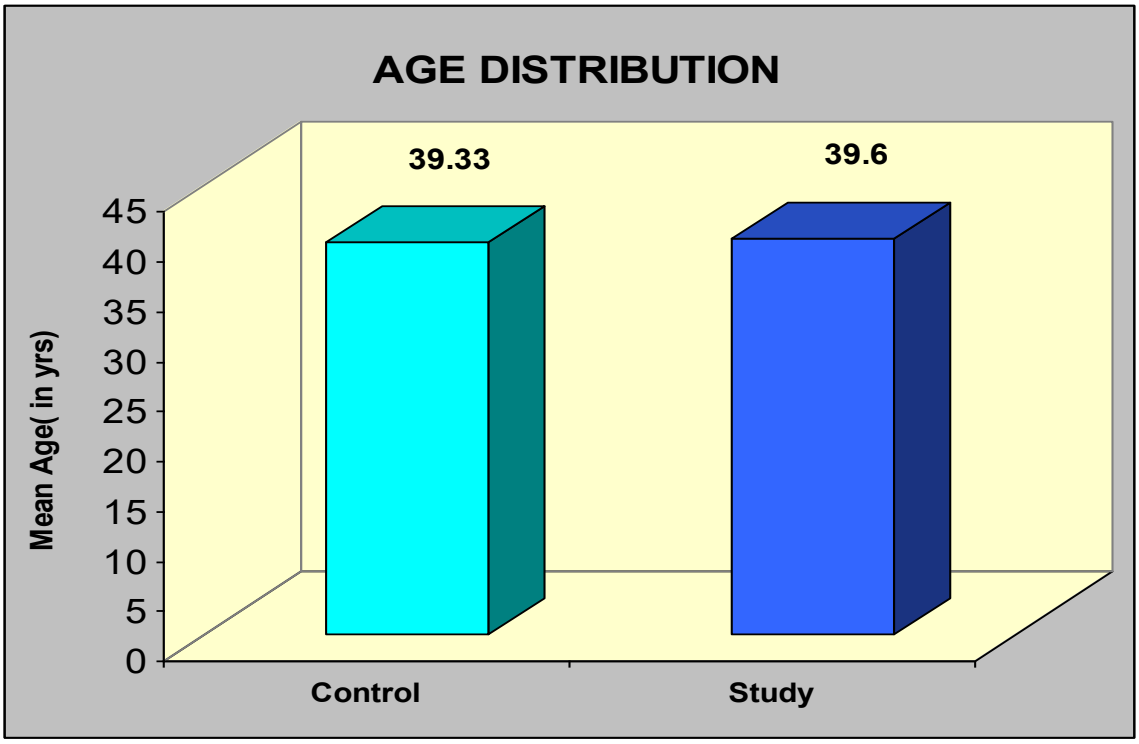


Figure 1 shows the diagrammatic representation of the age distribution in the study and the control groups.

SEX DISTRIBUTION

Table 2 :

		Group				Chi square test
		Control		Study		
		n	%	n	%	
Sex	Male	9	22.5%	8	20.0%	$\chi 2=0.08$ P=0.77
	Female	31	77.5%	32	80.0%	
Total		40	100.0%	40	100.0%	

There was no statistically significant difference among the groups regarding sex distribution.

Figure 2 :

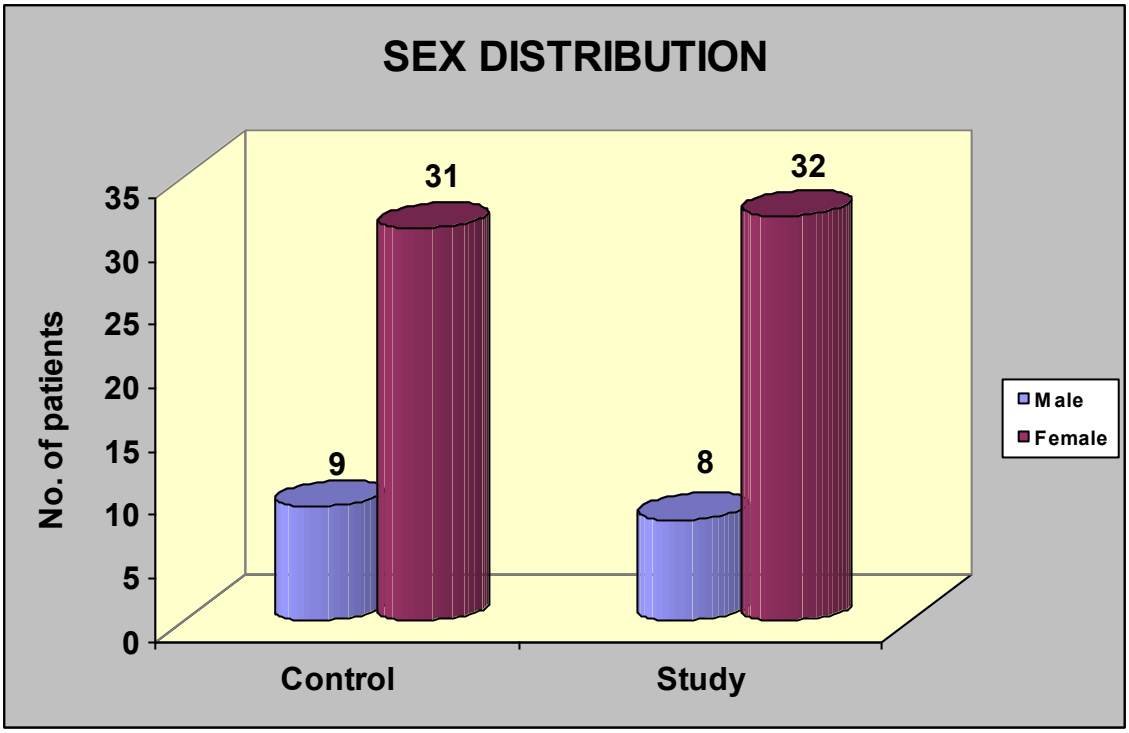


Figure 2 shows the bar diagram of sex distribution among the study and the control groups.

ASTHMA CONTROL QUESTIONNAIRE SCORE (ACQ)

Table 3 :

	ACS (Baseline)		ACS (2 weeks)		ACS (4 weeks)		ACS (6 weeks)		ACS (8 weeks)		ACS (12 weeks)		Oneway ANOVA F-test	Repeated Measures of ANOVA
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Control	21.30	5.18	21.08	5.26	20.90	5.38	20.40	5.26	19.83	5.08	19.70	5.38	t=1.66 P=0.20	Between groups F=3.06P=0.05*
Study	20.90	6.13	20.58	6.26	19.50	6.40	18.02	6.35	16.68	6.42	17.00	6.49	t=15.92. P=0.001*	Within group F=169.17* P=0.001
Student independent t-test	t=0.31 P=0.75		t=0.38 P=0.70		t=1.06 P=0.29		t=2.01* P=0.05		t=2.43* P=0.02		t=2.03* P=0.05			

*Significant

There was no statistically significant difference among the groups at the baseline, and at the end of the 2^{nd} and 4^{th} week, while there was a statistically significant difference in the study group when compared with the control group at the end of the 6^{th}, 8^{th} and 12^{th} week.

Figure 3 :

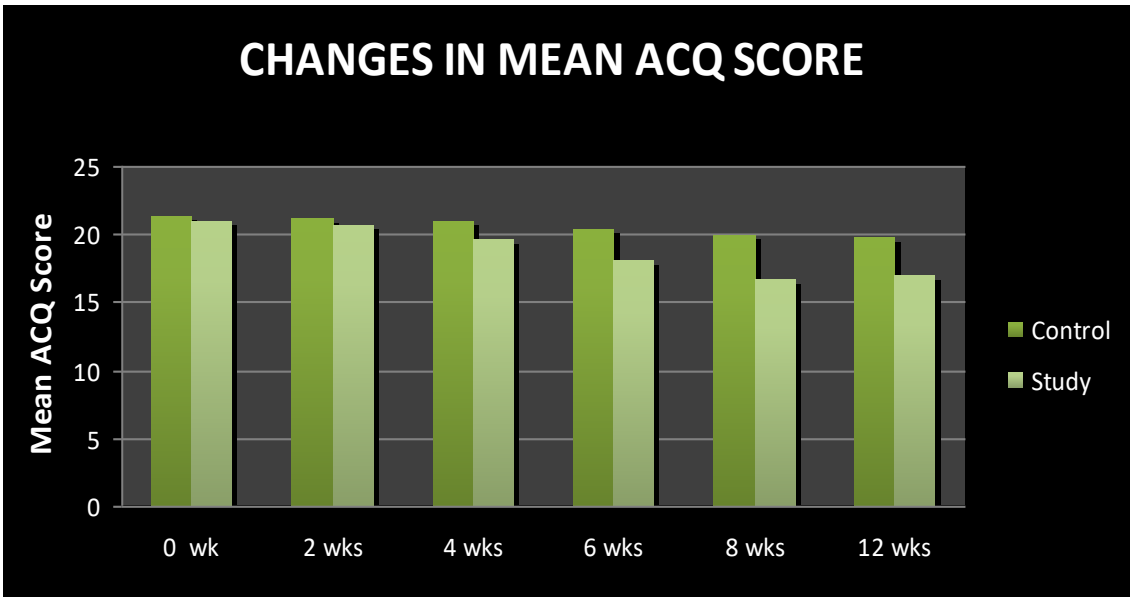
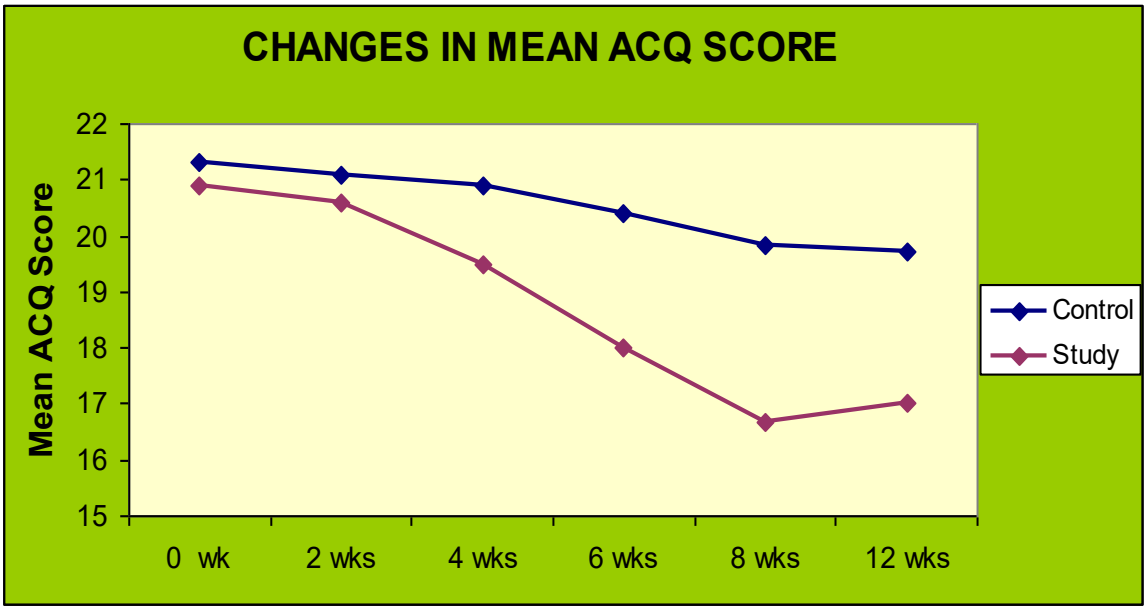


Figure 4 :



Figures 3 and 4 show the diagrammatic representation of the Asthma Control Questionnaire score in the study and the control groups at the baseline and at the end of the 2nd, 4th, 6th, 8th and 12th week.

PEAK EXPIRATORY FLOW RATE

Table 4 :

	PEFR (Baseline)		PEFR (2 weeks)		PEFR (4 weeks)		PEFR (6 weeks)		PEFR (8 weeks)		PEFR (12 weeks)		Oneway ANOVA F-test	Repeated Measures of ANOVA
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Control	278.00	77.96	283.25	77.74	288.00	76.60	293.00	76.60	298.25	75.78	296.50	79.28	t=0.82 P=0.36	Between groups F=4.08P=0.05*
Study	279.50	84.52	295.75	84.00	305.00	87.47	330.00	80.19	334.50	84.55	325.00	93.42	t=13.46. P=0.001*	Within group F=17.34* P=0.01
Student independent t-test	t=1.54 P=0.13		t=0.90 P=0.36		t=0.30 P=0.76		t=2.11* P=0.04		t=2.86* P=0.01		t=1.47 P=0.14			

*Significant

There was no statistically significant difference among the groups at the baseline, and at the end of the 2nd and 4th week, while there was a statistically significant difference in the study group when compared with the control group at the end of the 6th, 8th and 12th week.

Figure 5 :

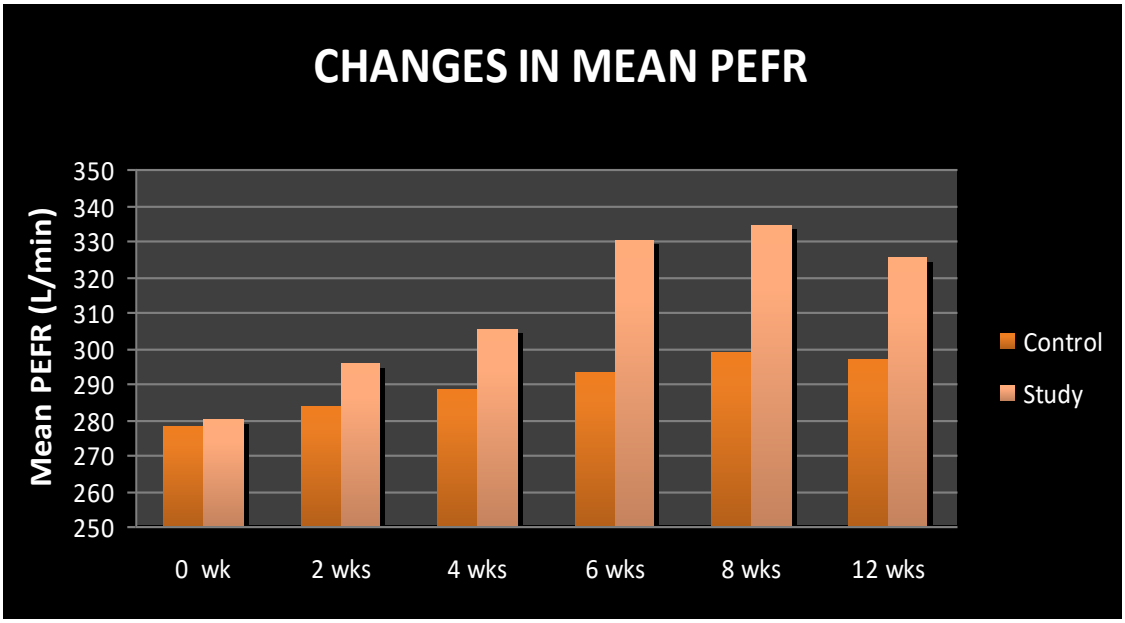
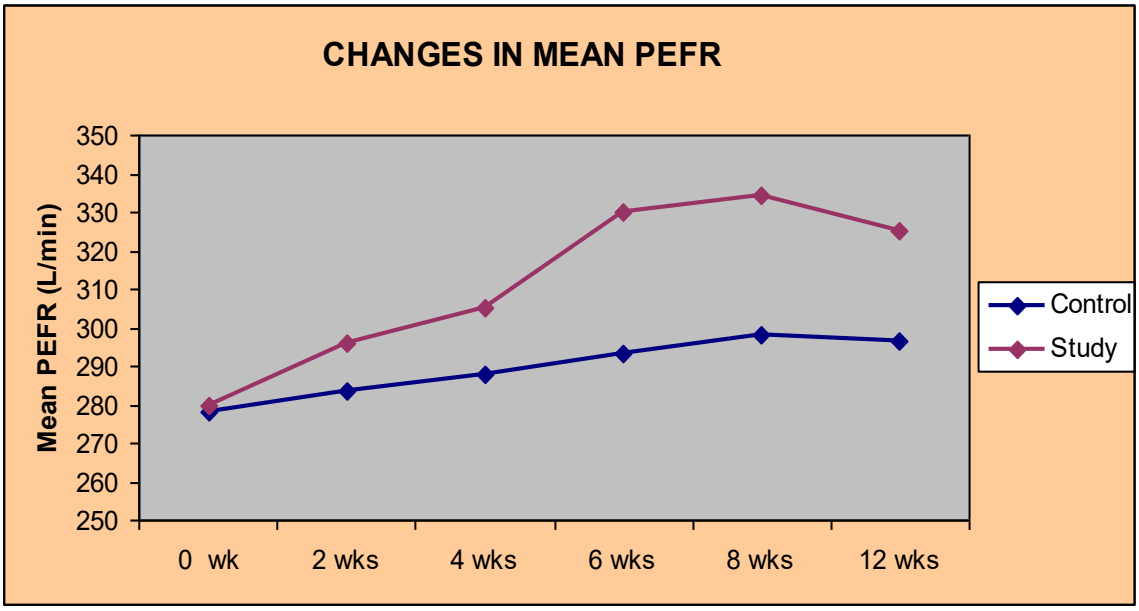


Figure 6 :



Figures 5 and 6 show the diagrammatic representation of Peak expiratory flow rate at the baseline and at the end of the 2nd, 4th, 6th, 8th and 12th week among the study and the control groups.

FORCED EXPIRATORY VOLUME IN ONE SECOND (FEV_1)

Table 5 :

	FEV_1 (Baseline)		FEV_1 (4 weeks)		FEV_1 (8 weeks)		FEV_1 (12 weeks)		Oneway ANOVA F- test	Repeated Measures of ANOVA
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Control	67.5	14.67	68.55	14.44	70.13	14.25	69.95	14.47	F=1.17 P=0.56	Between groups F=6.02 P=0.01*
Study	68.43	15.96	72.53	15.47	77.88	14.47	76.25	14.38	F=14.56 P=0.001*	Within group F=22.27* P=0.001
Student Independent t-test	t=1.05 P=0.74		t=1.18 P=0.23		t=2.22 P=0.02*		t=1.96 P=0.05*			

*Significant

There was no statistically significant difference among the groups at the baseline and at the end of the 4^{th} week, while there was a statistically significant difference in the study group when compared with the control group at the end of the 8^{th} and 12^{th} week.

Figure 7 :

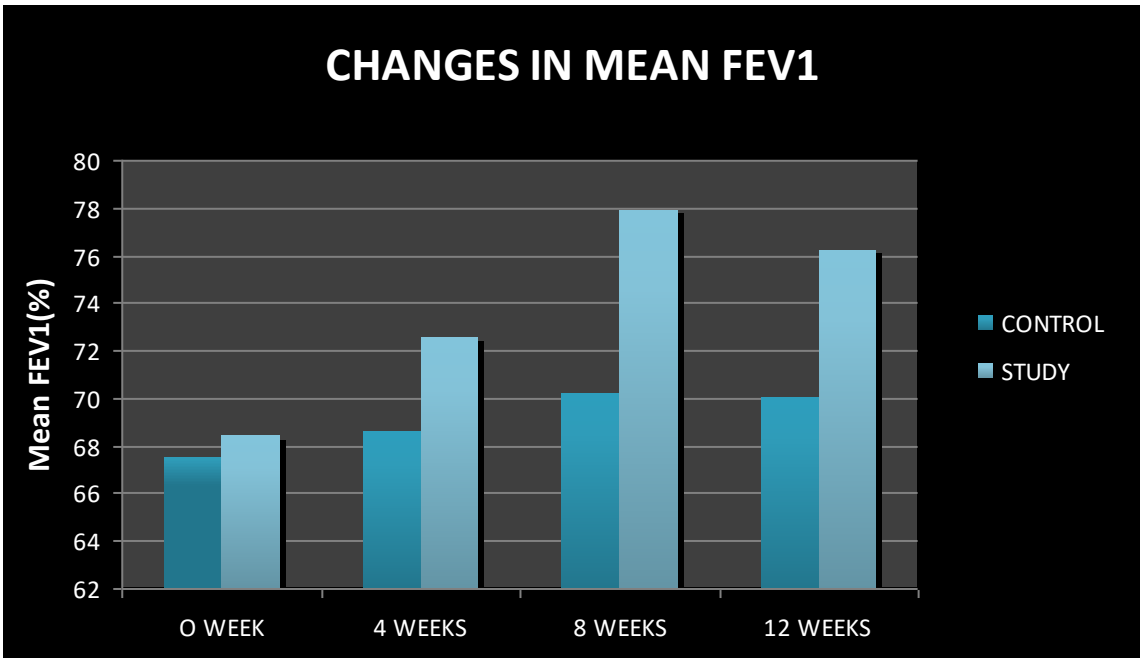
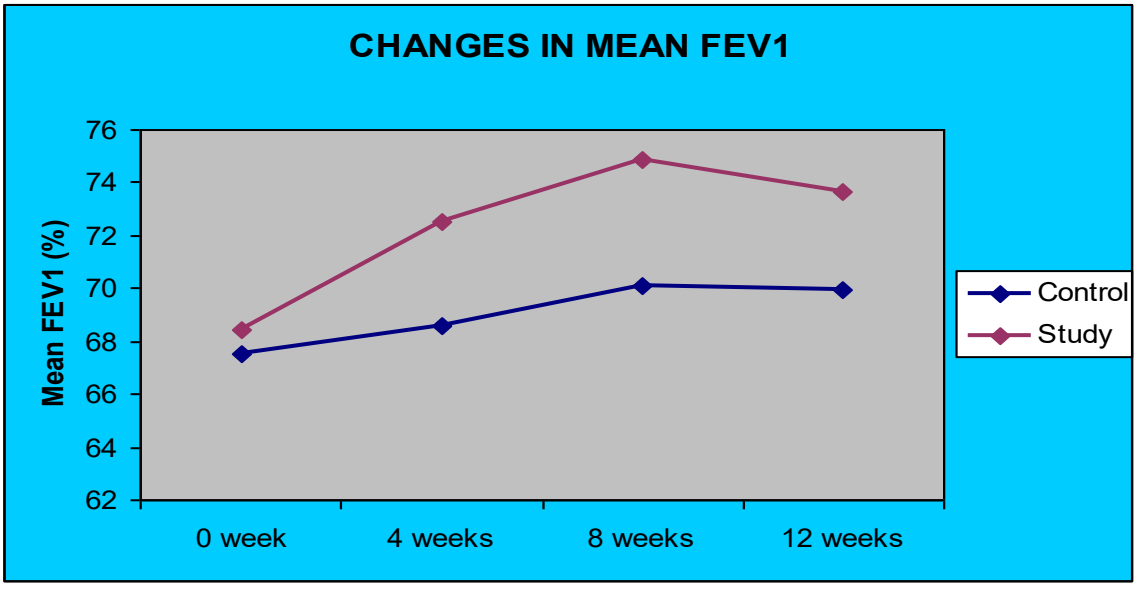


Figure 8 :



Figures 7 and 8 show the diagrammatic representation of the changes in the mean FEV_1 of the study and the control groups at the baseline and at the end of the 4^{th}, 8^{th} and 12^{th} week.

Changes in mean serum Malondialdehyde (MDA) level

Table 6 :

Group		Mean	Std. Deviation	Student paired t-test
Control	MDA 1 (Baseline)	23.18	4.242	t=0.84 P=0.41
	MDA 2 (8 Weeks)	23.38	3.946	
Study	MDA 1 (Baseline)	24.70	6.981	t=4.46 P=0.001*
	MDA 2 (8 Weeks)	23.13	5.923	

There was a statistically significant decrease in the mean serum MDA level in the study group when compared to the control group.

Figure 9 :

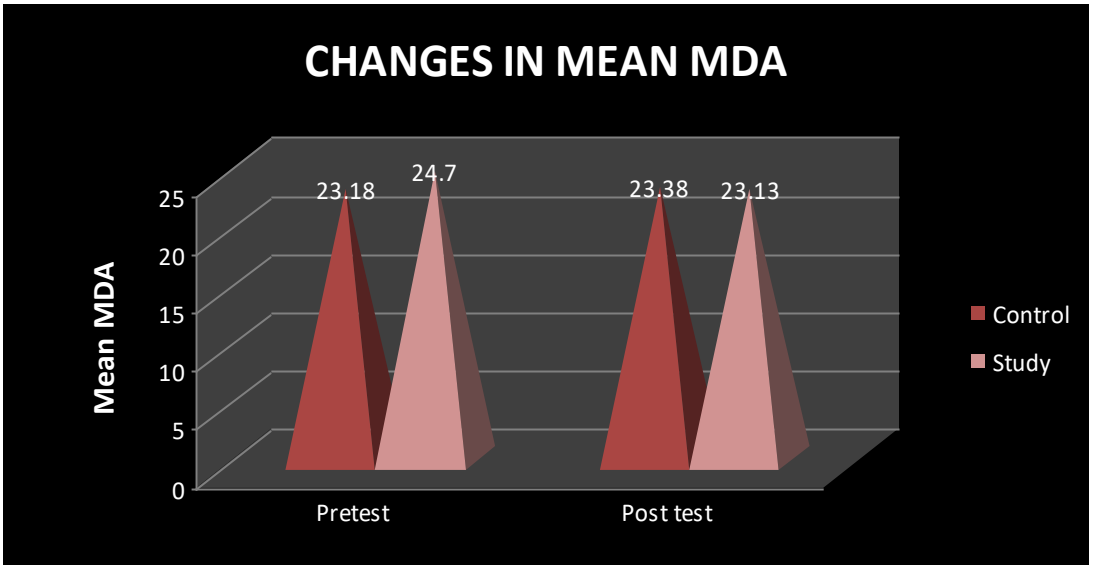
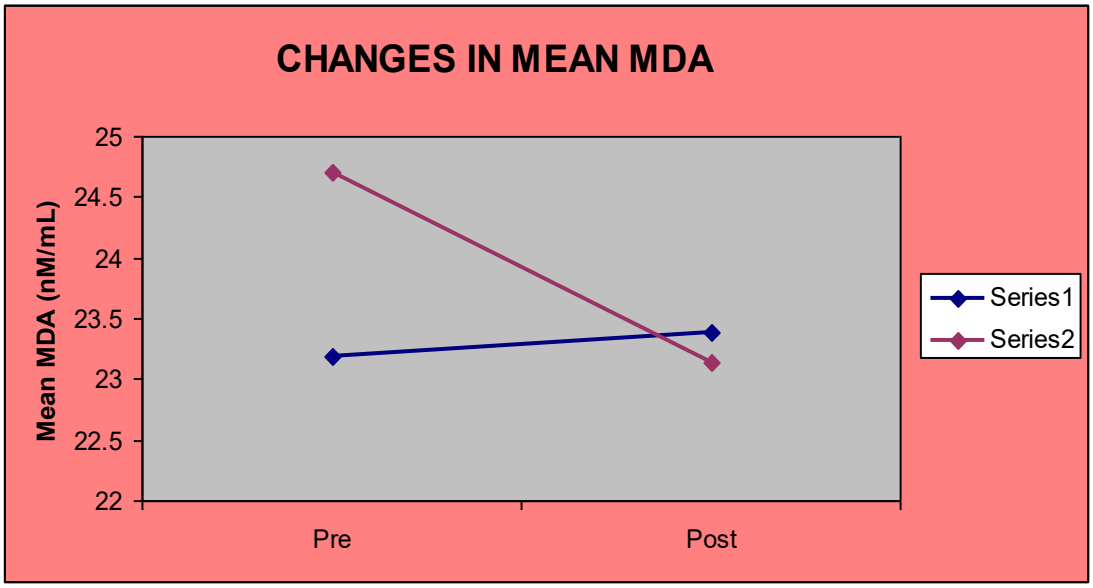


Figure 10 :



Figures 9 and 10 show the diagrammatic representation of the changes in the mean serum MDA level in the study and the control groups.

Changes in mean C-reactive protein level

Table 7 :

Group		Mean	Std. Deviation	Student paired t-test
Control	CRP 1 (Baseline)	8.25	5.207	t=1.71 P=0.10
	CRP 2 (8 Weeks)	9.60	5.891	
Study	CRP 1 (Baseline)	8.40	5.401	t=1.86 P=0.07
	CRP 2 (8 Weeks)	6.75	5.118	

There was no statistically significant difference in the mean CRP level among both the groups before and after the study period.

Figure 11 :

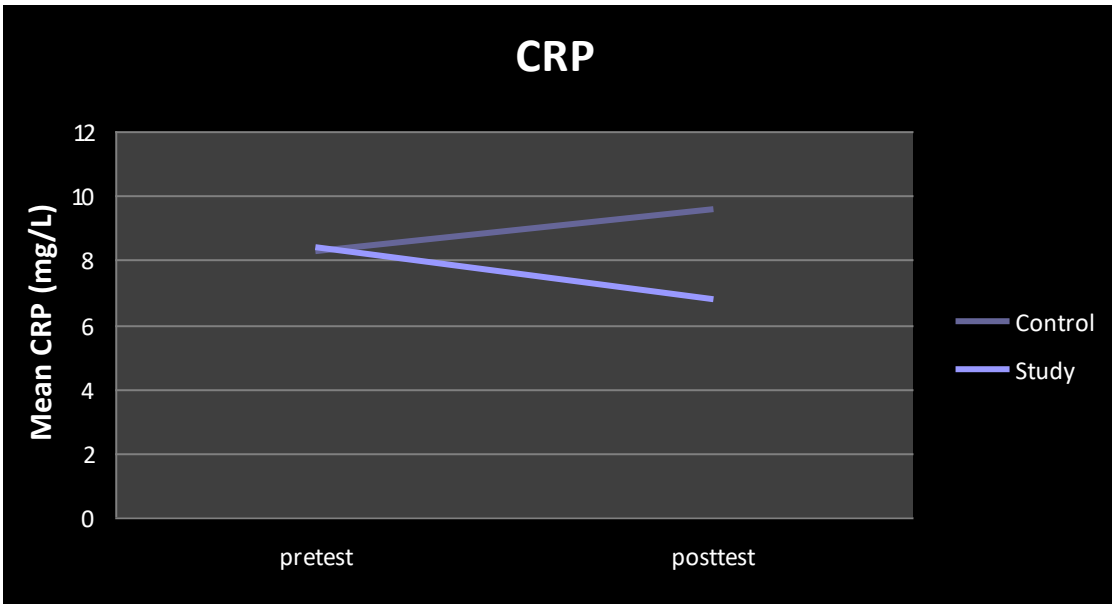
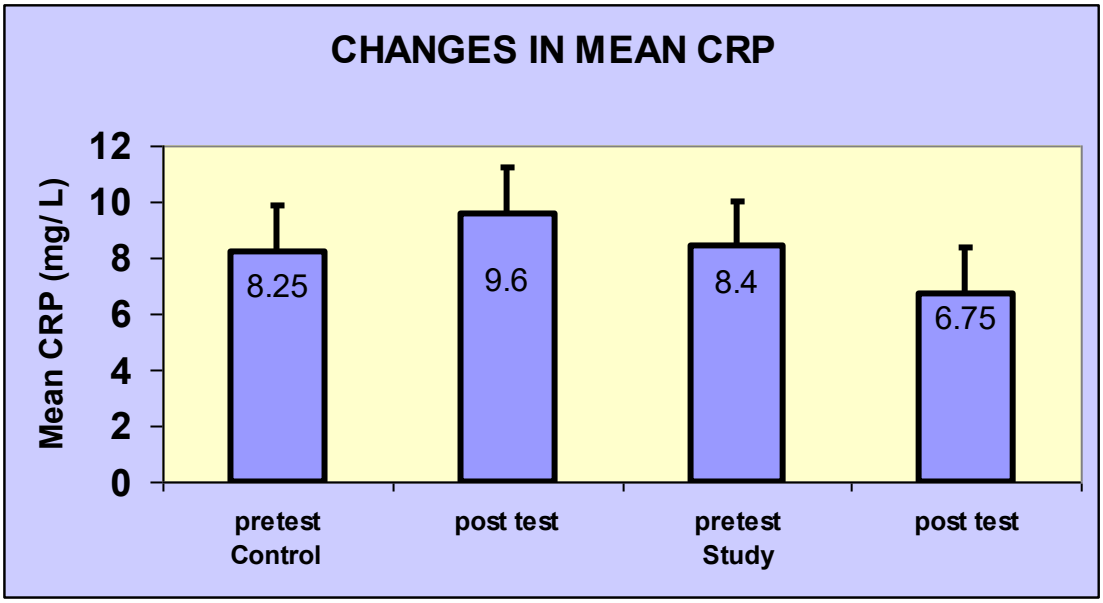


Figure 12 :



Figures 11 and 12 show the diagrammatic representation of the changes in the mean C-reactive protein level in the study and the control groups before and after the study period.

Percentage of reduction/increase in ACS, PEFR and FEV_1

Table 8 :

S.No	Parameters	% of reduction /increase in scores	
		Control Group	Study Group
1	ACS	- 7.5%	- 20.2%
2	PEFR	7.59%	19.68%
3	FEV_1	3.59%	13.81%

Figure 13 :

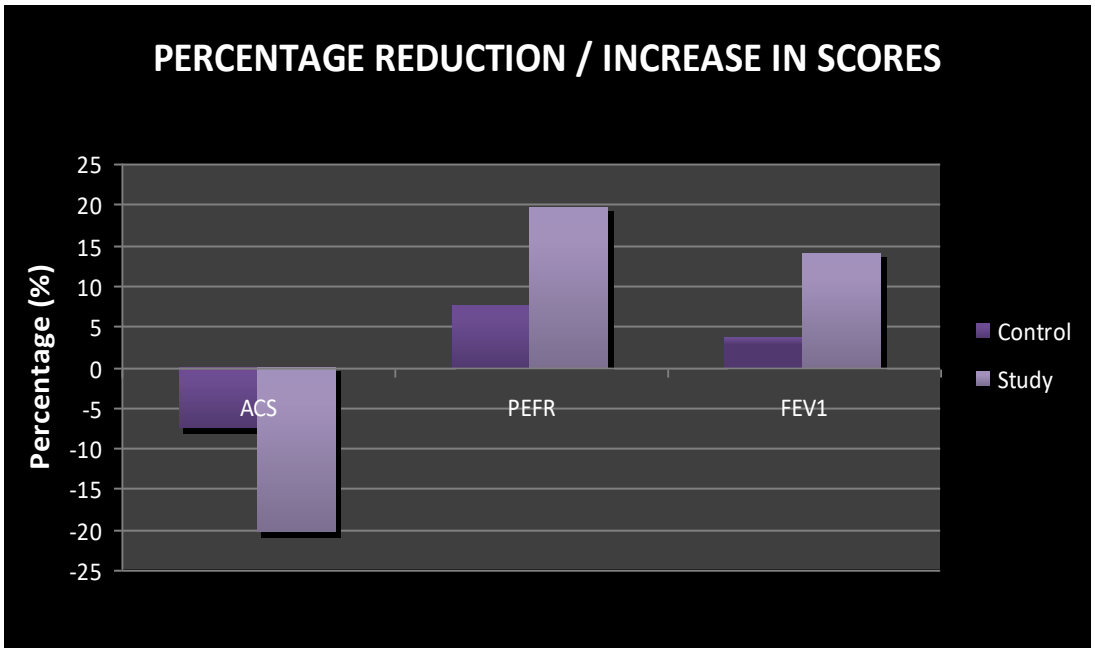
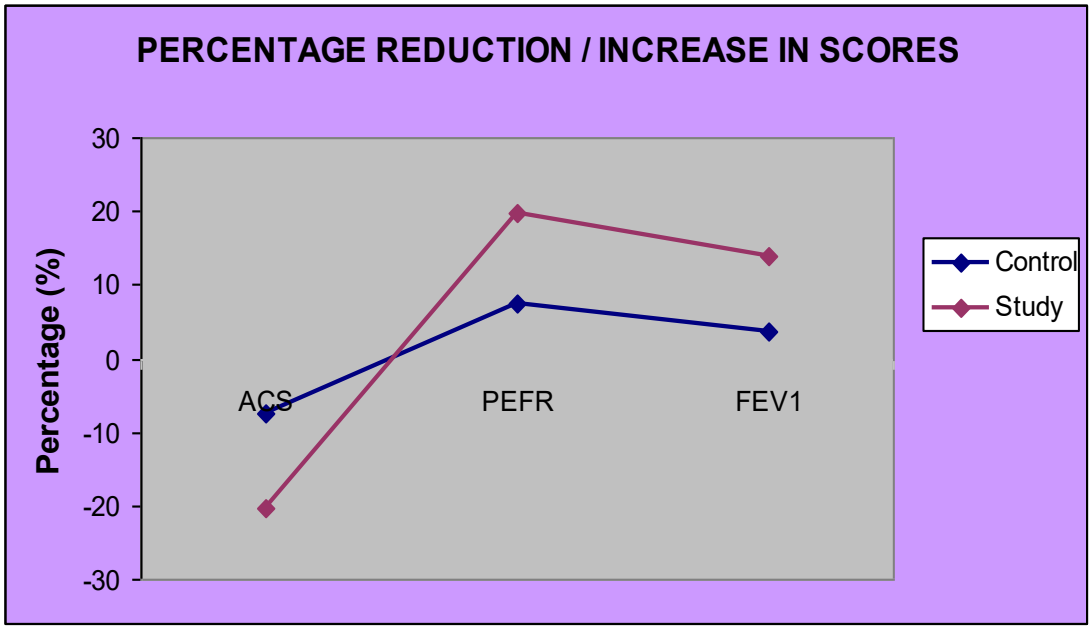


Figure 14 :

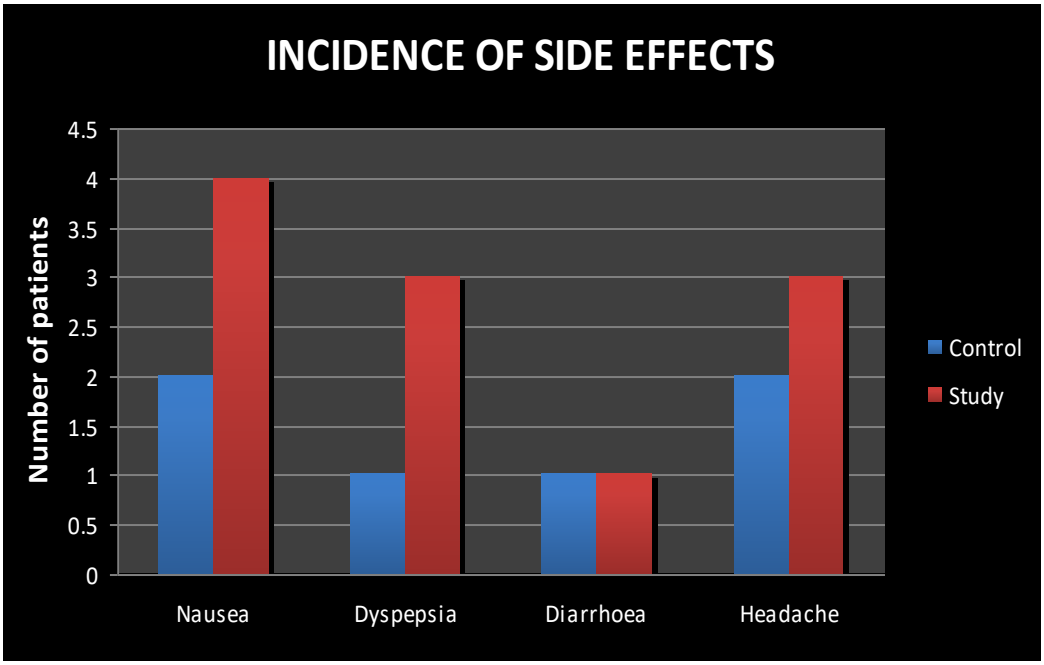


Incidence of side effects

Table 9 :

S.No	Side Effects	Control Group	Study Group
1.	Nausea	2	4
2.	Dyspepsia	1	3
3.	Diarrhoea	1	1
4.	Headache	2	3

Figure 15 :



DISCUSSION

This 12-week randomized, active controlled, prospective study examined the beneficial effects of Vitamin C supplementation in patients with chronic bronchial asthma.

The results of our study showed that administration of Vitamin C as an add on therapy had a significant effect in reducing the morbidity, improving the lung function, and the quality of life in asthmatics.

Out of the 112 patients screened, 85 patients who fulfilled the inclusion criteria were recruited for the study.They were randomized into control and study groups, 42 and 43 in number respectively.There were 5 dropouts, 2 from the control group and 3 from the study group.40 patients from the control group and 40 patients from the study group completed the study and were included in the statistical analysis.None of the dropouts were due to adverse effects.

The majority of patients in this study were in the age group of 30-50 years with high prevalence among females (vide tables 1 and 2, pictures 1 and 2).This was in correlation with the established demographic reports (Sally E Wenzel et al, 2000).

Efficacy variables such as ACQ score and PEFR were measured at the baseline and at the end of the 2^{nd}, 4^{th}, 6^{th}, 8^{th}, and 12^{th} week, while FEV_1 was measured at the baseline and at the end of the 4^{th}, 8^{th} and 12^{th} week.

Serum Malondialdehyde (MDA-a marker of oxidant injury) and Serum C – reactive protein (CRP- a marker of inflammation) were measured at the baseline and

at the end of the 8th week.Other routine haematological and biochemical parameters were done before and after the study.

ACQ SCORE

Clinically, asthma control was assessed using the Juniper Asthma Control Questionnaire (ACQ) score.ACQ was developed to optimise asthma control and thus reduce the risk of life threatening exacerbations and long term morbidity.It measures both the adequacy of asthma control and change in asthma control, which occurs either spontaneously or as a result of treatment.

From our study, it was evident that Vitamin C was effective in lowering the ACQ score as shown by a decline in nocturnal symptoms, severity of asthma attack, limitation of activities, shortness of breath and frequency of asthma attack (vide table 3, pictures 3 and 4).This was in correlation with the results of Sandra L.Tecklenburg et al, 2007 who showed that ascorbic acid supplementation significantly improved the ACQ score[75].

LUNG FUNCTION TESTS

Lung function parameters like Peak expiratory flow rate (PEFR) and Forced expiratory volume in one second (FEV_1) were significantly increased in the study group when compared to the control group (vide tables 4 and 5, pictures 5, 6, 7 and 8).These results were consistent with the data obtained from the studies of AR Ness and colleagues et al, 1996 at the Institute of Public Health,Cambridge,UK[76] who reported that Vitamin C is protective for lung function as evidenced by a positive correlation between plasma Vitamin C and FEV_1.This was supported by Schatcher and Schlesinger et al, 2001 who studied the effects of ascorbic acid in exercise

induced asthma and concluded that ascorbic acid supplementation has a protective effect on Peak expiratory flow rate (PEFR), Forced expiratory volume in one second (FEV_1) and Forced vital capacity (FVC) when compared to placebo[77].

SERUM MALONDIALDEHYDE

It is an established fact that the etiopathogenesis of asthma is related to excessive free radical formation.Free radicals are continuously produced in the body mostly by biochemical redox reactions involving oxygen, which occurs as a part of normal cell metabolism.

Oxidative stress occurs when there is an imbalance between the production and scavenging of free radicals, leading to oxidative deterioration of polyunsaturated fatty acids or lipids, thus compromising cellular function and the antioxidant status of the body.With excessive free radical production, there is an increase in lipid peroxidation as indicated by an increase in the levels of Serum Malondialdehyde (MDA), an aldehydic end product of lipid peroxidation.

Vitamin C exerts its antioxidant effect by curbing the excessive free radical formation and inhibiting the initiation of lipid peroxidation, thereby controlling the MDA levels.

In the present study, MDA was found to have higher values in both the groups at the start of the study (vide table 6, pictures 9 and 10).This was in correlation with the research done by Nadeem A Chhaba et al, 2003 who reported increased lipid peroxidation products and an increase in MDA levels, in patients with bronchial asthma [48].

There was a statistically significant decrease in the MDA levels in the study group at the end of the study which was not seen in the control group (vide table 6, pictures 9 and 10).This decrease is probably due to supplementation with Vitamin C which is able to counter the excess free radical production and thus inhibits lipid peroxidation .The results obtained in this study were comparable with the results of Luqman A Olayaki and Sabhu M Ajao et al, 2008 and Jaswal S Mehta HC et al, 2003 who showed a decrease in the concentration of MDA levels after antioxidant therapy[78,79].

SERUM C-REACTIVE PROTEIN

Chronic inflammation of the airway plays a major role in the pathogenesis of asthma which is associated with an increased plasma CRP level.CRP is used mainly as a marker of inflammation.Measuring and charting C – reactive protein values can prove useful in determining the disease progression or the effectiveness of treatment.This was supported by the research work done by Ahmed A Arif and George L Deluos et al, 2002 who showed that adults with asthma and asthma symptoms have higher levels of CRP[80].

In our study, there was a decrease in the CRP levels in the study group when compared to the control group which was not statistically significant (vide table 7, pictures 11 and 12).This may be due to the fact that the CRP levels in our study were estimated using the ELISA technique whereas a significant decrease could probably be appreciated only when measured using high sensitivity technique as reported by M Takemura and M Matsumoto[81] et al, 2006.

The other haematological and biochemical parameters measured before and after the study were found to have no statistical difference among both the groups.

Mild adverse effects such as nausea, dyspepsia, diarrhoea and headache occurred in both the groups.

FOLLOW UP

At the end of active drug therapy, patients were asked to report to the asthma clinic after 1 month for follow up.All patients, both in the control and study group were assessed clinically by using subjective and objective criteria.There was no change observed in the control group while there was a slight decline in the improvement observed in the study group which was not statistically significant (vide tables 3, 4 and 5, pictures 3, 4, 5, 6, 7 and 8).

Vitamin C is an important antioxidant in the extracellular respiratory lining fluid that protects proteases, antiproteases, epithelial and immune cells from oxidant attack and low levels of this antioxidant may leave the lungs relatively unprotected from oxidant stress.

As an antioxidant, Vitamin C's primary role is to neutralize free radicals.Since it is water soluble, Vitamin C can work both inside and outside the cells to combat free radical damage.As explained earlier, free radicals will seek out an electron to regain their stability.Vitamin C is an excellent source of electrons, therefore it can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity.In addition to its work as a direct scavenger of free radicals in fluids

and lipids, Vitamin C also contributes to the antioxidant activity of Vitamin E and glutathione.

Thus, from this study it is evident that oral administration of Vitamin C as an add on therapy produces significant improvement in asthma control and severity.

CONCLUSION

In conclusion, this study proves the role of Vitamin C in reducing the oxidant injury associated with chronic airway inflammation in asthma.

This study also shows that Vitamin C as an add on therapy to the existing standard therapy improves the clinical response and decreases the disease activity to a greater extent than with routine standard drug therapy alone.

In view of its low cost, safety and efficacy, the routine use of Vitamin C in chronic bronchial asthma warrants consideration.Further, the combination of Vitamin C with other known antioxidants like Vitamin A, Vitamin E, β carotene in achieving better asthma control can also be evaluated.

BIBLIOGRAPHY

1. J. A. Innes, P.T. Reid. Respiratory Diseases. Davidson's *Principles and Practice of Medicine*, 20th edn: 670-678; Nicholas A. Boon, Nicki R Colledge, Brian R Walker, John AA Hunter (eds); Churchill Livingstone Elsevier.

2. E. R. McFadden, Jr. Diseases of the Respiratory system. *Harrison's Principles of Internal Medicine*, vol 2, 15th edition: 1419-1420; Anthony S. Fauci, Dennis L. Kasper, Dan L. Longo, Eugene Braunwald, Stephen L. Hauser (eds); Mc-Graw Hill.

3. Sally E. Wenzel. Asthma, Disorders of the Respiratory System. *Kelley's Textbook of Internal Medicine*, 4th edition: 2427-2428.

4. Olumide Cole. Introduction to asthma. *Journal of the Association of Physicians of India* 2002; vol 50: 465-495.

5. Global Initiative for Asthma (GINA); *Global strategy for asthma management and prevention.* Bethasda: National Institute of Health, National Heart, Lung and Blood Institute. NIH Publication No 02-3659, 2006.

6. Juniper, O'Byrne PM, Guyatt, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999; 14(4): 902-907.

7. Gardiner PV, Ward C, Booth H, Allison A, Hendrick DJ, Walters EH. Effect of eight weeks of treatment with salmetrol on bronchoalveolar lavage inflammatory indices in asthmatics. *Am J Respir Crit Care Med* 1994; 150: 1006-1011.

8. O'Byrne PM, Bisgaard H, Godard PP, Pisolesi M, Palmqvist M, Zhu Y, et al. Budesonide/formoterol combination therapy as both maintenance and reliever medication in asthma. *Am J Respir Crit Care Med* 2005; 171: 129-36.

9. British Thoracic Society/SIGN. British guideline on the management of asthma. *Thorax* 2003; 58 (supplement1): 1-83.

10. Ayres JG, Higgins B, Chilvers ER, Ayre G, Blogg M, Fox H. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with poorly controlled (moderate-to-severe) allergic asthma. *Allergy* 2004; 59: 701-708.

11. Duthie GG. Fat Soluble Vitamins. *Human Nutrition and Dietetics*, 10th edition: 226-236; Garrow, James, Ralph (eds); Churchill Livingstone.

12. Lee SH, Oe T, Blair IA. Vitamin C-induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science* 2001; 292: 2083-2086.

13. Barnes PJ. Reactive Oxygen Species and Airway Inflammation. *Free Rad Biol Med* 1990; 9: 235-243.

14. Doelman CJA, Bast A. Oxygen radicals in lung pathology. *Free Rad Biol Med* 1990; 9: 381-400.

15. Paredi P, Kharitonov SA, Barnes PJ. Elevation of exhaled ethane concentration in Asthma. *Am J Respir Crit Care Med* 2000; 162; 1450-1454.

16. Levine SJ. Bronchial epithelial cell-cytokine interactions in airway inflammation. *J Invest Med* 1995; 43: 241-249.

17. Neyens HJ, Raatgeep RE, Degenhart HJ, Duiverman EJ, Kerrebign KF.Altered Leucocyte response in relation to the basic abnormality in children with asthma and bronchial hyperresponsiveness. *Am Rev Respir Dis* 1984; 130: 744-747.

18. Cluzel M, Damon M, Chanez P, et al. Enhanced alveolar cell luminol-dependent Chemiluminescence in asthma. *J Allergy Clin Immunol* 1987; 80: 195-201.

19. Jarjour NN, Calhoun WJ. Enhanced production of oxygen radicals in asthma. *J Lab Clin Med* 1994; 123: 131-136.

20. Abeles AM, Pillinger MH. Neuropeptides, Free Radicals and Nitric Oxide. *Rheumatology*, vol 1, 3rd edn; 140-144. Hochberg, Silman, Smolen, Weinblatt, Weismann (eds); Mosby Elsevier.

21. Steudel W, Hurford E, Zapol WM. Inhaled Nitric Oxide, Basic Biology and Clinical Applications. *Anaesthesiology* 1999; 91 (4): 1090-1112.

22. Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howrath P, Redington A et al. Induction of NO synthase in asthma. *Lancet* 1993; 342: 1510-1513.

23. Giaid A, Saleh D, Lim S, Barnes PJ, Ernst P. Formation of peroxynitrite in asthmatic airways. *FASEB J* 1998; 12: 929-937.

24. Kharitonov SA, Yates D, Barnes PJ. Regular inhaled budesonide decreases Nitric Oxide concentration in the exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996; 153: 454-457.

25. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled NO, sputum eosinophils and methacholine responsiveness. *Thorax* 1998; 53; 91-95.

26. Rhoden KJ, Barnes PJ. Effect of hydrogen peroxide on guinea pig tracheal muscle in vitro; role of cyclooxygenase and airway epithelium. *Br J Pharmacol* 1989; 98: 325-330.

27. Katsumata U, Miura M, Ichinose M, et al. Oxygen radicals produce airway constriction and hyperresponsiveness in anaesthetized cats. *Am Rev Respir Dis* 1990; 141: 1158-1161.

28. Weiss EB, Bellino JR. Leukotriene-associated toxic oxygen metabolites induces airway hyperreactivity. *Chest* 1986; 89: 709-716.

29. Adler KB, Holden-Stauffer WJ, Repine JE. Oxygen metabolites stimulate release of high molecular weight glycoconjugates by cell and organ cultures of rodent respiratory epithelium via arachidonic acid dependent mechanism. *J Clin Invest* 1990; 85: 75-85.

30. Phipps RJ, Denas SM, Sielczak MV, Wanner A. The effect of 0.5ppm ozone on glycoprotein secretion, ion and water fluxes in sheep trachea. *J Appl Physiol* 1986; 60: 918-927.

31. Doelman CJA, Leurs R, Oosterom WC, Bast A. Mineral dust exposure and free radical-mediated lung damage. *Exp Lung Res* 1990; 16: 41-55.

32. Del Maestro RF, Bjork J, Arfors K. Increase in microvascular permeability induced by enzymatically generated free radicals. I. Invivo study; *Microvasc Res* 1981; 22: 239-254.

33. Tate RM, Van Benthuysen KM, Shasby DM, McMurtry IF, Repine JE. Oxygen-radical-mediated permeability edema and vasoconstriction in isolated perfused rabbit lungs. *Am Rev Respir Dis* 1982; 126: 802-806.

34. Biagioli MC, Kaul P, Singh I, Turner RB.The role of oxidative stress in Rhinovirus induced elaboration of IL-8 by respiratory epithelial cells. *Free Rad Biol Med* 1999; 26: 454-462.

35. Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Garg ML. Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. *Lipids* 2000; 35: 967-974.

36. Dworski R, Murray JJ, Roberts LJ 2nd, Oates JA, Morrow JD, Fisher L, Sheller JR. Allergen-induced synthesis of F2-isoprostanes in atopic asthmatics; Evidence for oxidant stress. *Am J Respir Crit Care* Med 1999; 160: 1947-1951.

37. Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. *Eur Respir J* 2003; 21: 177-186.

38. Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD and smokers. *Am J Respir Crit Care Med* 1996; 154: 1055-1060.

39. Kalayci O, Besler T, Kilinc K, Sekerel BE, Saraclar Y. Serum levels of antioxidant vitamins in children with bronchial asthma. *Turkish J Pediatr* 2000; 42: 17-21.

40. Shanmugasundaram KR, Kumar SS, Rajajee S. Excessive free radical generation in the blood of children suffering from asthma. *Clinical Chimica Acta* 2001; 305; 107-114.

41. Ozarus R, Tahan V, Turkmen S, et al. Changes in malondialdehyde levels in bronchoalveolar fluid and serum in the treatment of asthma with inhaled steroid and beta2-agonist. *Respirology* 2000; 5; 289-292.

42. Antczak A, Nowak D, Shariati B, Krol M, Piasecka G, Kurmanowska Z. Increased hydrogen peroxide and thiobarbituric acid-reactive products in expired breath condensate of asthmatic patients. *Eur Respir J* 1997; 10: 1235-1241.

43. Olopade CO, Zakkar M, Swedler WI, Rubinstein I; Exhaled pentane levels in acute asthma. *Chest* 1997; 111: 862-865.

44. Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol* 2002; 110: 349-356.

45. Dworski R. Oxidative stress in asthma. *Thorax* 2000; 55(supplement 2): S51-53.

46. Henricks PA, Nijkamp FP. Reactive oxygen species as mediators in asthma. *Pulm Pharmacol Ther* 2001; 14: 409-420.

47. Rahman I, MacNee W. Reactive oxygen species. In: Barnes PJ, Drazen J, Rennard S, et al, eds. *Asthma and COPD*. London: Academic press, 2002: 243-254.

48. Nadeem A, Chhabra SK, Masood A, et al. Increased oxidative stress and altered levels of antioxidants in asthma. *J Allergy Clin Immunol* 2003; 111: 72-78.

49. Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998; 50: 515-596.

50. Smith LJ, Shamsuddin M, Sporn PHS, et al. Reduced superoxide dismutase in lung cells of patients with asthma. *Free Radic Biol Med* 1997; 22: 1301-1307.

51. De Raeve HR, Thunnissen FBJM, Kaneko FT, et el. Decreased Cu, Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroids in vivo. *Am J Physiol* 1997; 272: 115-118.

52. Hulsmann AR, Raatgeep HR, Den Hollander JC, et al. Oxidative epithelial damage produces hyperresponsiveness of human peripheral airways. *Am J Respir Crit Care Med* 1994; 149: 519-525.

53. Kanazawa H, Shiraishi S, Hirata K,et al. Decreased peroxynitrite inhibitory activity in induced sputum in patients with bronchial asthma. *Thorax* 2002; 57: 509-512.

54. Puddicombe SM, Torres-Lozano C, Richter A, et al. Increased expression of p21 (waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. *Am J Respir Cell Mol Biol* 2003; 28: 61-68.

55. Comhair SA, Bhathena PR, Farver C, et al. Extracellular glutathione peroxidase induction in asthmatic lungs: evidence for redox regulation of expression in human airway epithelial cells. *FASEB J* 2001; 15: 70-78.

56. Omland O, Deguchi Y, Sigsgaard T, et al. Selenium serum and urine is associated to mild asthma and atopy. The SUS study. *J Trace Elem Med Biol* 2002; 16: 123-127.

57. Papi A, Padadopoulos NG, Stanciu LA, et al. Reducing agents inhibit rhinovirus-induced up-regulation of the rhinovirus receptor intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells. *FASEB J* 2002; 16: 1934-1936.

58. Biagioli MC, Kaul P, Singh I, et al. The role of oxidative stress in rhinovirus induced elaboration of IL-8 by respiratory epithelial cells. *Free Radic Biol Med* 1999; 26: 454-464.

59. Smit H, Grievink L, Ocke M, Veer P, Kromhout D. Dietary intake of antioxidant vitamins,respiratory symptoms and pulmonary function. *Thorax* 1998; 53 (3): 166-171.

60. Cho YS, Lee J, Lee TH, Lee EY, Lee KU, Park JY, Moon HB. α-Lipoic acid inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. *J Allergy Clin Immunol* 2004; 114: 429-435.

61. Ford ES, Mannino DM, Redd SC. Serum antioxidant concentrations among U.S. adults with self-reported asthma. *J Asthma* 2004; 41: 179–187.

62. Gary E Hatch. Asthma, inhaled oxidants, and dietary antioxidants. *Am J Clinical Nutrition* 1995; 61: 625S-630S.

63. Buettner GR, Moseley PL. EPR spin trapping of free radicals produced by bleomycin and ascorbate. *Free Rad Res Commun* 1993; 19: S89-S93.

64. Harakeh S, Jariwalla R, Pauling L. Suppression of human immunodeficiency virus replication by ascorbate in chronically and acutely infected cells. *Proceedings of National Academy of Sciences, USA* 1990; 87 (18): 7245-7249.

65. Simon JA, Hudes ES. Relationship of ascorbic acid to blood lead levels. *JAMA* 1999; 281 (24): 2289-2293.

66. Dawson E, Evans D, Harris W, Teter M, McGanity W. The effect of ascorbic acid supplementation on the blood levels of smokers. *J Am Coll nutrition* 1999; 18 (2): 166-170.

67. Akmal M, Qadri J, Al-Waili N, Thangal S, Haq A, Saloom K. Improvement in human semen quality after oral supplementation of Vitamin C. *Journal of medicinal food* 2006; 9 (3): 440-442.

68. Enstrom JE, Kanim LE, Klein MA. *Epidemiology* 1992; 3: 194-202.

69. Osganian SK, Stamfer MJ, Rimm E, et al. *J Am Coll Cardiol* 2003; 42: 246-252.

70. Janet Wittes et al. Age related eye disease study. *Arch Ophthalmol* 2001; 119: 1417- 1436.

71. Nathens A, Neff M, Jurkovich G, Klotz P, Farver K, et al. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. *Annals of Surgery* 2002; 236 (6): 814-822.

72. Mohammad Al Biltagi, Baset AA, Bassiouny M, Mohammad Al Kasrawi, Attia M. Omega-3 fatty acids, Vitamin C, Zn supplementation in asthmatic children: a randomized self-controlled study. *Acta paediatrica* 2009; 98 (4): 737-742.

73. Jo Leonardi Bee, Allen S, Britton JR. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. *Thorax* 2009; 64: 610-619

74. Jaber R et al. Respiratory and allergic diseases: from upper respiratory tract infections to asthma. *Prim Care* 2002; 29 (2): 231-261.

75. Sandra L Tecklenburg, Timothy D Mickleborough, Alyce D Fly, Yeon Bai, Joel M Stager. Ascorbic acid supplementation attenuates exercise induced bronchoconstriction in patients with asthma. *Respiratory Medicine* 2007; 101: 1770-1778.

76. Ness AR, Khaw KT, Bingham S, Day NE. Vitamin C status and respiratory function. *Eur J Clin Nutrition* 1996; 50(9): 573-579.

77. Schatcher EN, Schlesinger A. The attenuation of exercise-induced bronchospasm by ascorbic acid. *Ann Allergy* 1982; 49: 146-151

78. Luqman A Olayaki, Salihu M Ajao, Gafar A Jimoh,Ayodele O Soladoye. Effect of vitamin c on malondialdehyde in pregnant nigerian women. *J Bas Appl Sciences* 2008; 4 (2): 105-108.

79. Jaswal S, Mehta HC, Sood AK, Kaur J. Antioxidant status and the role of antioxidant therapy. *Clin Chim Acta* 2003; 338: 1-2: 123-129

80. Ahmed A Arif, George L Deluos, Jane Colmer H. Association between asthma, asthma symptoms and C-reactive protein in US adults. *Respirology* 2007; 12 (5): 675-682.

81.Takemura M, Matsumoto H, Niimi A, Ueda T, Matsuoka H, Yamaguchi M, et al. High sensitivity C-reactive protein in asthma. *Eur Respir J* 2006; 27: 908-912.

ANNEXURE - I

CERTIFICATE FOR APPROVAL OF ETHICAL COMMITTEE

To

Dr.K.G.Devibala , PG in M.D(Pharmacology)

Dear **Dr.K.G.Devibala , PG in M.D(Pharmacology)**

The Institutional Ethics Committee reviewed and discussed your application for approval of the project entitled

"Evaluation of the role of vitamin C in bronchial asthma"

The following members of the ethics committee were present at the meeting held on 09.06.2008 at the Modernised Seminar Hall, Stanley Medical College, Chennai-1 at 10.00AM

Dr.A.Sundaram Dean i/c -
Chairman and Member Secretary of the Ethics Committee

MEMBERS

Dr.Jayanthi
Prof.of Medical Gastroenterology
Dr.Madhavan
Prof.of Pharmacology
Dr.Rengaramani
Prof.of Biochemistry
Dr.Madhan
Prof.of Aneasthesiology
Dr.Thenmozhivalli
Prof.of Microbiology

We approve the project to be conducted in its presented form.

The Institutional Ethics Committee/Independent Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

Yours sincerely,

Member Secretary,
Ethics Committee

STANLEY MEDICAL COLLEGE
17 6 2008
CHENNAI-600 001

ANNEXURE – II

ஒப்புதல்

திரு/ திருமதி /செல்வி ..... ஆகிய நான், இந்த மருத்துவ சோதனைக்கு உட்பட மனப்பூர்வமாக ஒப்புதல் தெரிவிக்கிறேன். இந்த மருத்துவ ஆய்வின் நோக்கம் ஆஸ்துமா என்னும் நோய்க்கு விட்டமின் சி என்னும் மாத்திரையை உட்கொள்வதால் ஏற்படும் நன்மைகளை பற்றி அறிவது ஆகும் என்று தெரிந்து கொண்டேன். இந்த மருத்துவ பரிசோதனையில் சில கேள்விகளுக்கு பதில் அளிக்கவும், இங்கே சேகரிக்கப்பட்ட அனைத்து விவரங்களும், எந்தவொரு அறிவியல் கூடத்திலும் தெரிவிக்கலாம் என்பதையும் தெரிந்து கொண்டேன்.

எந்தவொரு சூழ்நிலையிலும் இந்த ஆய்விலிருந்து விலகிக் கொள்ளலாம் என்பதையும், அதன் பின்னரும் எந்தவித இடையூறுமின்றி மருத்துவ சிகிச்சையை இந்த மருத்துவமனையில் தொடர்ந்து மேற்கொள்ளலாம் என்பதையும் புரிந்து கொண்டேன்.

கையொப்பம்

முதன்மை ஆய்வாளர்.

ANNEXURE - III

MASTER CHART

NAME : **DATE :**

AGE : **S.NO :**

SEX : **OP NO :**

ADDRESS :

PRESENT HISTORY :

PAST HISTORY :

PREVIOUS MEDICATIONS AND DETAILS :

EXAMINATION

I. GENERAL EXAMINATION :

II. CVS :

III. RS :

IV. ABDOMEN :

I. ASTHMA CONTROL QUESTIONNAIRE SCORE

S.NO	SYMPTOMS	SCORE						
1	**NOCTURNAL SYMPTOMS**	0	1	2	3	4	5	6
2	**SEVERITY OF ASTHMA ATTACKS**	0	1	2	3	4	5	6
3	**LIMITATION OF ACTIVITIES**	0	1	2	3	4	5	6
4	**SHORTNESS OF BREATH**	0	1	2	3	4	5	6
5	**FREQUENCY OF ASTHMA ATTACK**	0	1	2	3	4	5	6
6	**SHORT-ACTING BRONCHODILATOR USE**	0	1	2	3	4	5	6
7	**FEV_1 / PEFR**	0	1	2	3	4	5	6

II. LAB INVESTIGATIONS

A. CBC

B. RANDOM BLOOD SUGAR

C. SERUM CREATININE

D. URINE ANALYSIS

E. SERUM C- REACTIVE PROTEIN

	AT BASELINE	AFTER 8 WEEKS
CRP		

F. SERUM MALONDIALDEHYDE

	AT BASELINE	AFTER 8 WEEKS
MDA		

III. PEAK EXPIRATORY FLOW RATE

	AT BASELINE	AFTER 2 WEEKS	AFTER 4 WEEKS	AFTER 6 WEEKS	AFTER 8 WEEKS	AFTER 12 WEEKS
PEFR						

IV. FORCED EXPIRATORY VOLUME IN ONE SECOND

	AT BASELINE	AFTER 4 WEEKS	AFTER 8 WEEKS	AFTER 12 WEEKS
FEV_1				

ANNEXURE - IV

ASTHMA CONTROL QUESTIONNAIRE SCORE

Circle the number that best describes how your asthma has been during this week.

1. On a average, during the past week, how often were you **woken by your asthma** during the night?

Never	0
Hardly ever	1
A few times	2
Several times	3
Many times	4
A great many times	5
Unable to sleep because of asthma	6

2. On a average, during the past week, how **bad were your asthma symptoms when you woke** up in the morning?

No symptoms	0
Very mild symptoms	1
Mild symptoms	2
Moderate symptoms	3
Quite severe symptoms	4
Severe symptoms	5
Very severe symptoms	6

3. In general, during the past week, how **limited were you in your activities** because of your asthma?

Not limited at all	0
Very slightly limited	1
Slightly limited	2
Moderately limited	3
Very limited	4
Extremely limited	5
Totally limited	6

4. In general, during the past week, how much **shortness of breath** did you experience because of your asthma?

None	0
A very little	1
A little	2
A moderate amount	3
Quiet a lot	4
A great deal	5
A very great deal	6

5. In general, during the past week, how much of the time did you **wheeze**?

Not at all	0
Hardly any time at all	1
A little of the time	2
A moderate amount of the time	3
A lot of the time	4
Most of the time	5
All the time	6

6. On average, during the past week,how many **puffs of short-acting bronchodilator** have you used each day?

None	0
1-2 puffs most days	1
3-4 puffs most days	2
5-8 puffs most days	3
9-12 puffs most days	4
13-16 puffs most days	5
More than 16 puffs most days	6

To be completed by a member of the clinic staff
(Record actual values in the shaded cells and score the FEV_1 % predicted in the last column)

FEV_1 prebronchodilator		> 95% predicted	0
		95-90%	1
FEV_1 predicted		89-80%	2
		79-70%	3
FEV_1 % predicted		69-60%	4
		59-50%	5
		< 50% predicted	6

TOTAL SCORE	
SCORE / 7	

ANNEXURE - V

DAILY DRUG REMINDER CHART

Days	Morning (8 am)	Evening (8 pm)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		

Please mark a tick (√) after taking the medicine, and mark (X) when medication is missed.
Please bring this chart at every visit.

Figure 1:

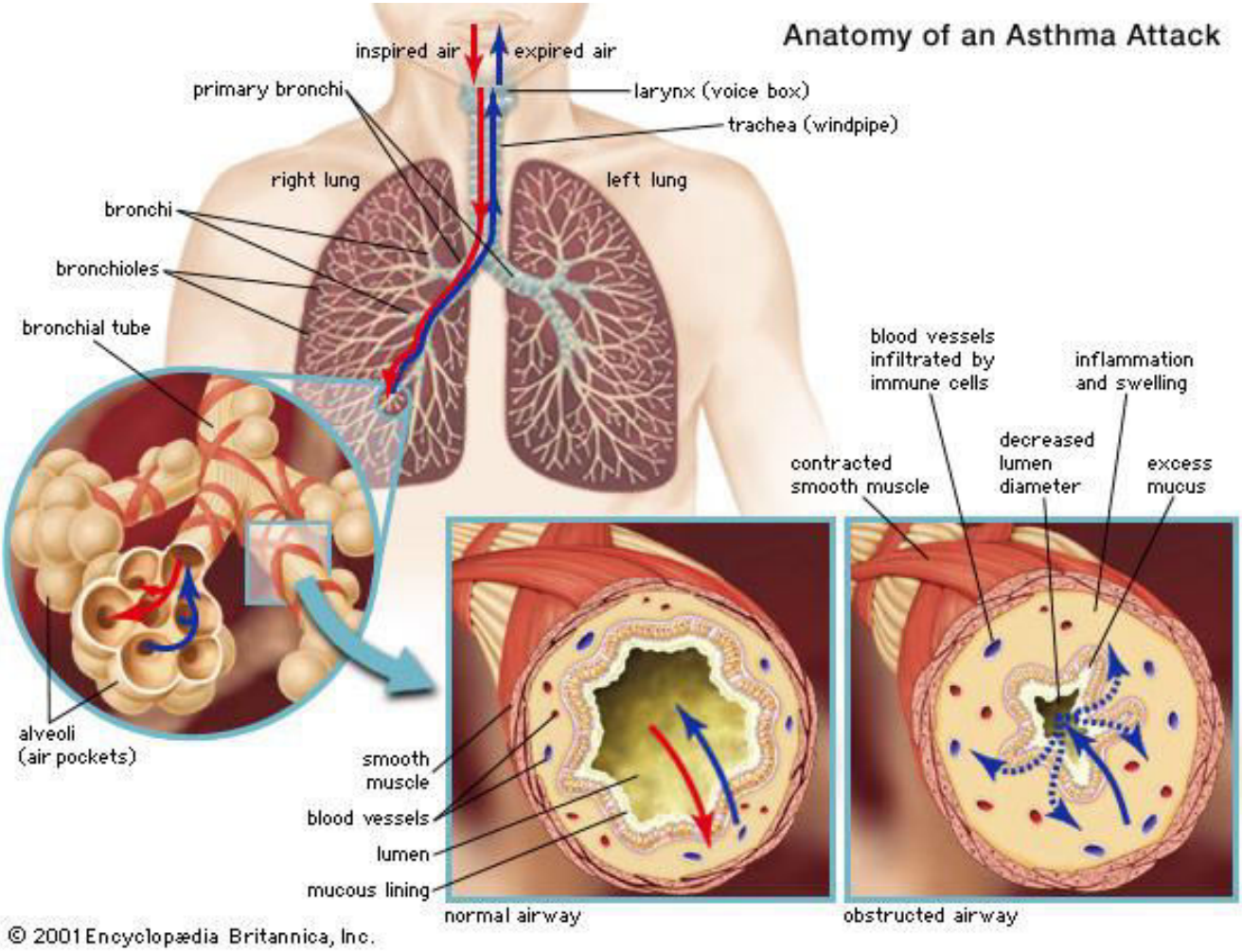
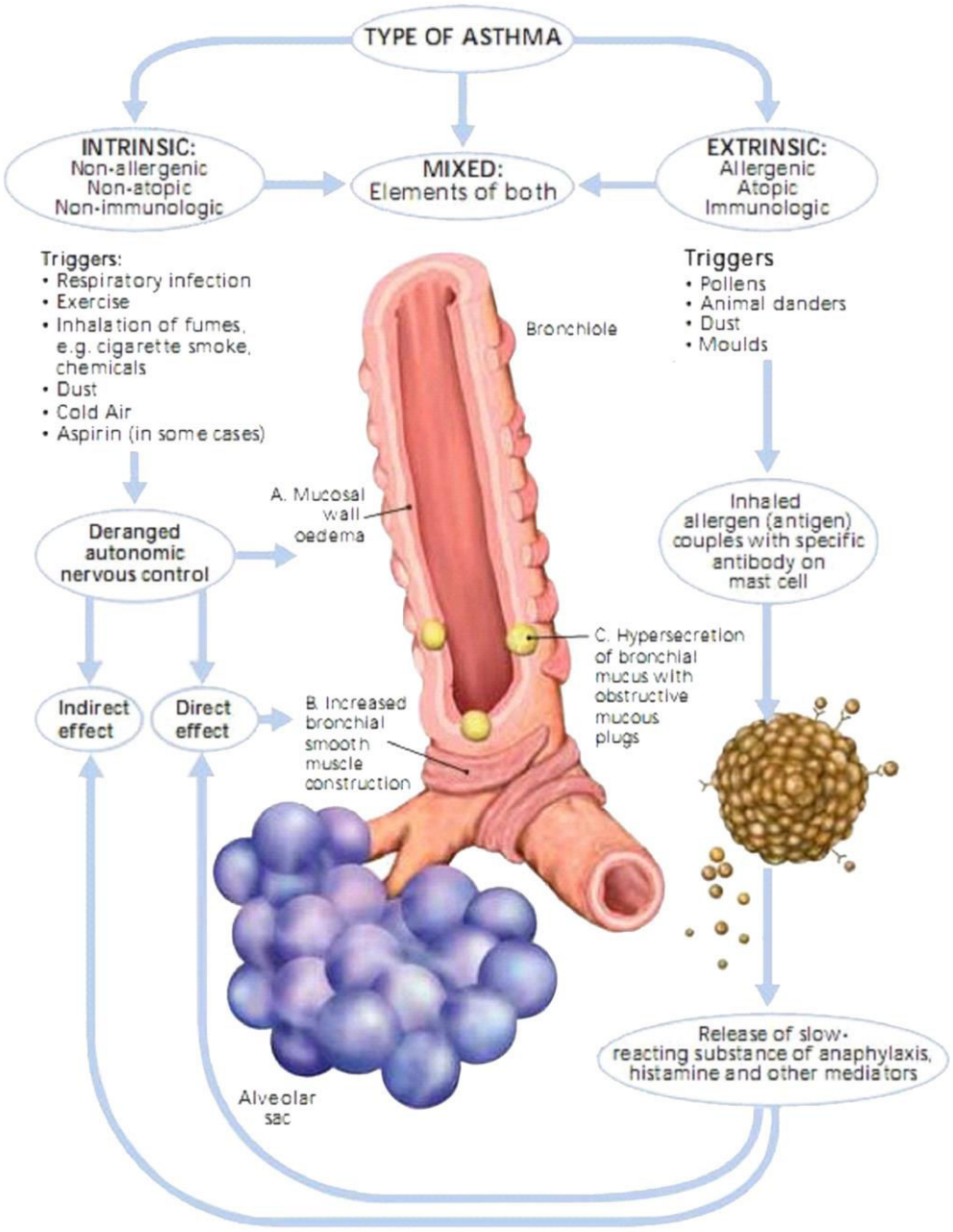
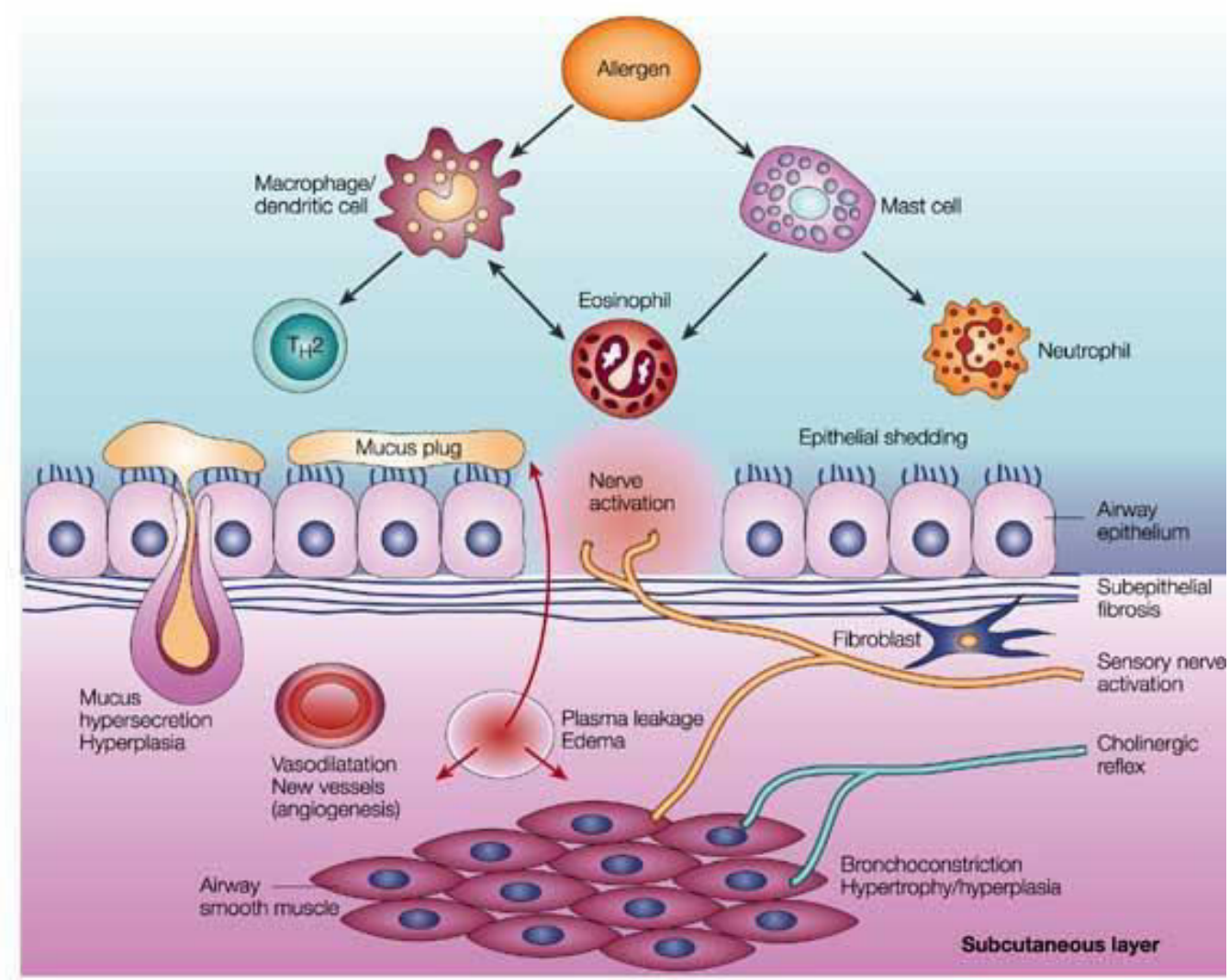

Anatomy of an Asthma Attack
inspired air
expired air
primary bronchi
larynx (voice box)
trachea (windpipe)
right lung
left lung
bronchi
bronchioles
bronchial tube
alveoli
(air pockets)
blood vessels
infiltrated by
immune cells
inflammation
and swelling
contracted
smooth muscle
decreased
lumen
diameter
excess
mucus
smooth
muscle
blood vessels
lumen
mucous lining
normal airway
obstructed airway
© 2001 Encyclopædia Britannica, Inc.

Figure 2 : Classification and Mechanism of asthma



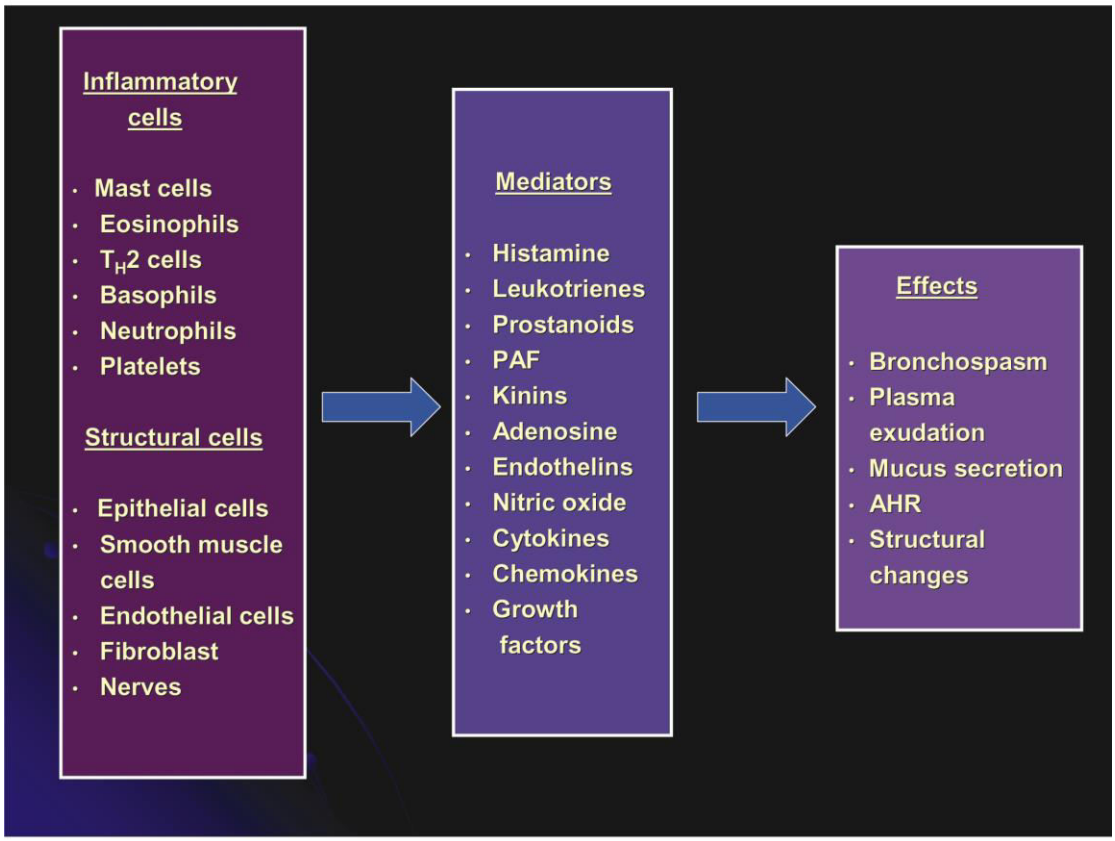
Source : Encyclopaedia of Natural Medicine

Figure 3 : Pathophysiology of asthma



Source : Harrison's Principles of Internal Medicine

Figure 4 : Inflammatory Cells and Mediators



Source : Harrison's Principles of Internal Medicine

Figure 5 : X-ray picture showing hyperinflation of lungs in asthma

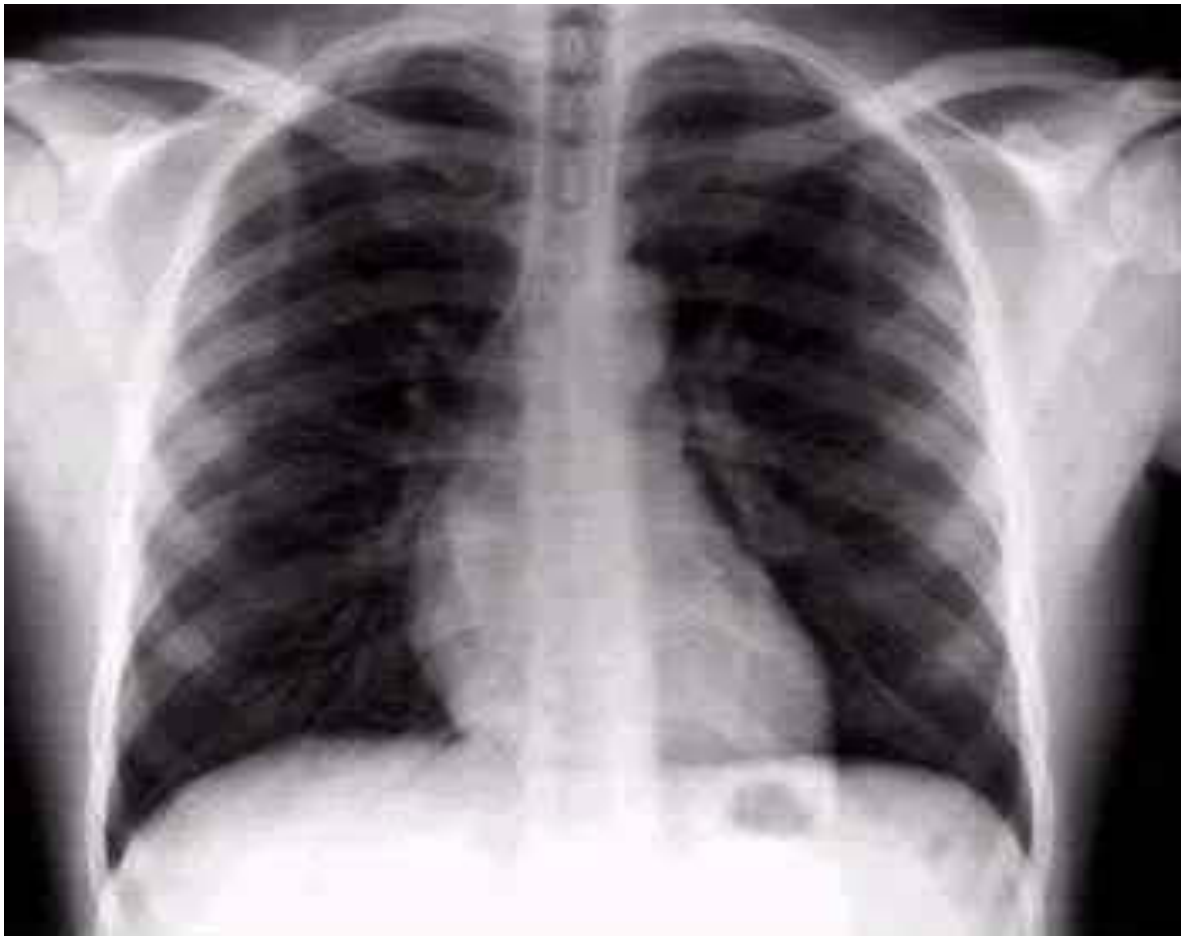


Figure 6 : Mechanism leading to lipid peroxidation in asthma

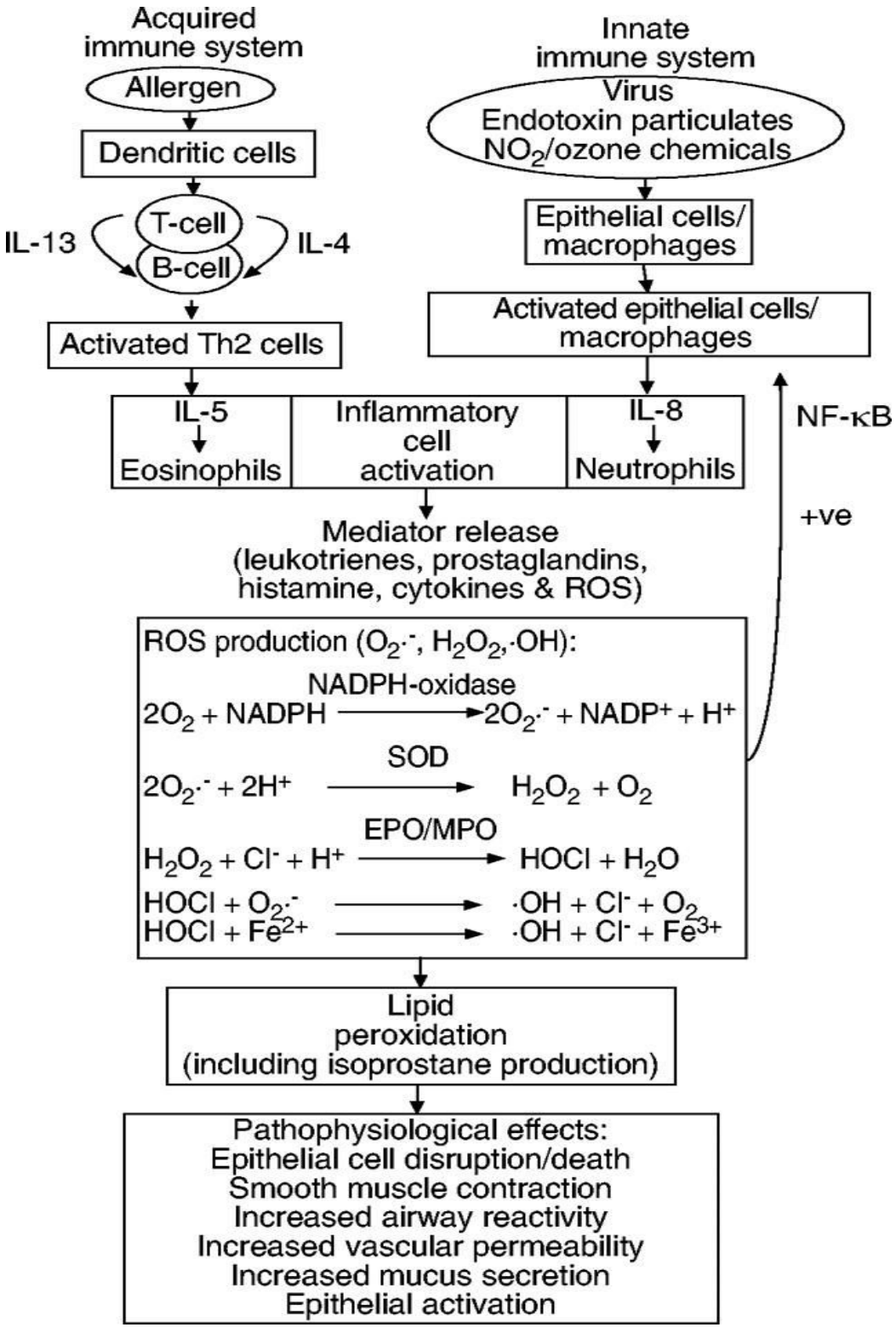
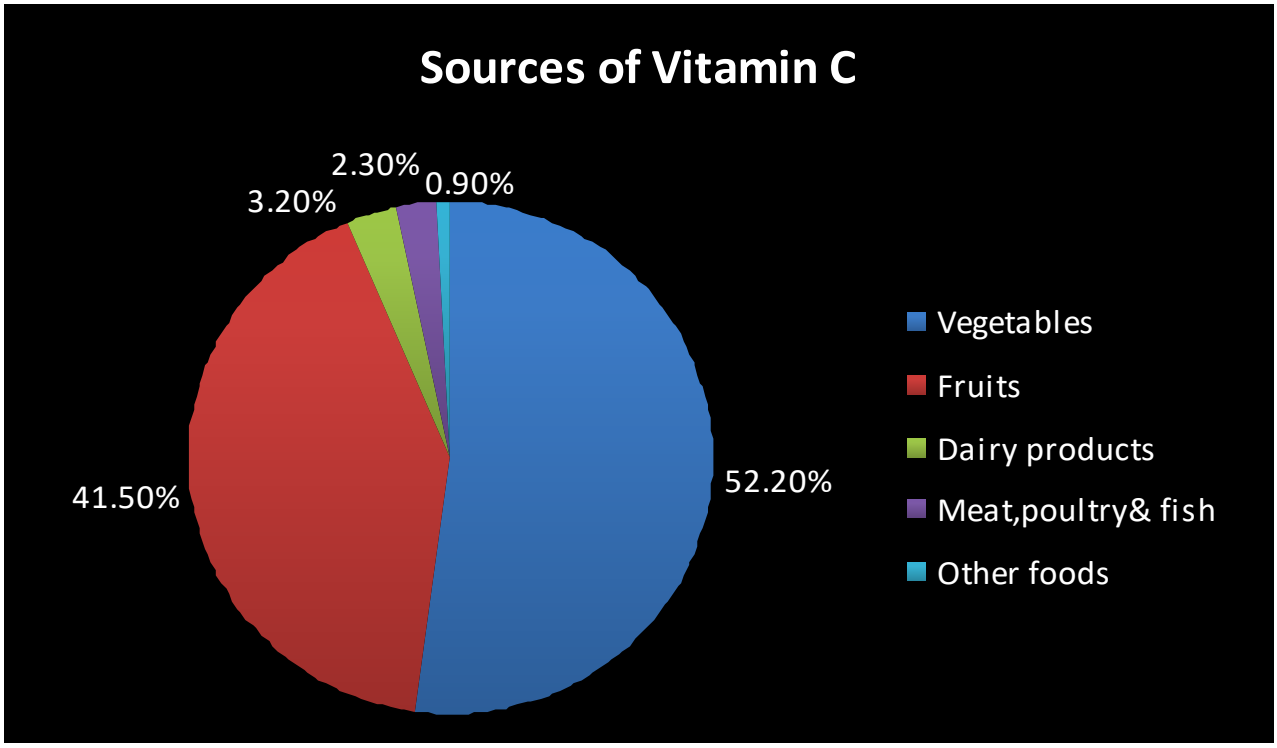
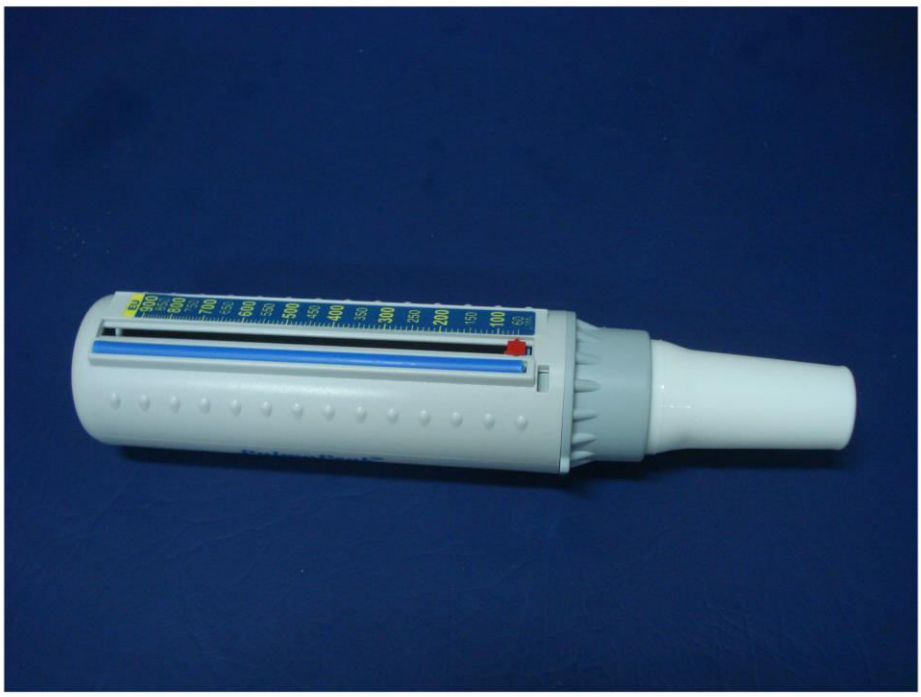


Figure 7 :


Sources of Vitamin C
2.30%
0.90%
3.20%
41.50%
52.20%
Vegetables
Fruits
Dairy products
Meat,poultry& fish
Other foods

PEAK EXPIRATORY FLOW METER



PHOTOGRAPH SHOWING PATIENT WITH PEAK EXPIRATORY FLOW METER

SPIROMETER

PHOTOGRAPH SHOWING PATIENT PERFORMING SPIROMETRY TESTING